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THE HEREDITARY FACTOR IN SOME DISEASES OF THE HAEMOPOIETIC SYSTEM

Lecture I of the William Sydney Thayer and Susan Read Thayer Lectures in Clinical Medicine (1928)[1]

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INTRODUCTION

It is indeed a great and happy honour to be asked to give these lectures named after the friend of many years—more, perhaps, than he and I may wish to count—the one in the United States I have been privileged to know longest. This Foundation is affectionately dedicated to William Sydney Thayer and Susan Read Thayer his wife, that gracious spirit, inseparable from him in the minds of their friends as a true counterpart to him who is left alone with the memory of "the light that was." Richly endowed with the charm and social gifts characteristic of the South, she freely gave her life first to the service of the suffering in the Johns Hopkins Hospital, then to humanitarian work in other directions and to seconding her husband in encouraging and guiding his pupils in the footsteps of the great clinicians. In the last stage of a long and trying illness the light of her nobility and unselfishness shone more brightly than even before.

With the changing fashions of thought, inevitable to the passing years, there has been some tendency to magnify the cleanly cut decisions of the laboratory at the expense of bedside experience, and to imagine that a clinical physician can be evolved with little trouble or time from the well drilled scientific student. It has been said that, like the poet, the clinical physician is born, not made, and no doubt

[1] The William Sydney Thayer and Susan Read Thayer Lectureship was established through the generosity of friends of Dr. and Mrs. Thayer. This is the first series of lectures to be given under this foundation. For an account of the presentation of the Thayer Lectureship to the University, see *Bulletin of the Johns Hopkins Hospital*, 1927, xli, no. 1,—Ed.

there is an element of truth in this, for some are certainly inherently unfit from the absence of the essential qualification of character and temperament. But this is not all; the fine and finished product results only from wide knowledge, including not only that of the laboratory but that of human nature, and long experience of the manifold aspects of disease. While a pathological training is essential, a man cannot at once blossom out into a clinical physician by the simple process of transferring his laboratory mind and methods from the pathological block to the wards. "There are few short-cuts in medicine; all the acquisitions of recent years have not shortened by a day the time that he who would be a skilled clinician must pass by the bedside."[2] An apprenticeship—longer than that of Jacob—must be served before what Robert Louis Stevenson called "the flower of our civilisation," the physician, can be brought to perfection. This ideal is admirably exemplified by Thayer's work, career, and influence, and should he ever be depicted, as many of the London physicians were in past times by "Spy," in Vanity Fair, the only appropriate legend below the cartoon would be "Clinical Medicine." It was a happy event in the medical history of the United States when at Washington, D. C., on 18 May, 1927, his friends and admirers presented this lectureship to the Johns Hopkins University, which he has served so long, so faithfully, and with such distinction. Of the numerous eponymous lectureships few, like this, have been founded during a man's active life, before "the sere, the yellow leaf," retirement, or complete rest from his labours have made a permanent memorial appropriate by the absence of the inspiring personality. There are exceptions to Thayer's own lines—

> 'Tis only those who're gone beyond recall
> That leave enduring shadows on life's wall.

In 1897 the Neurological Society of London established the Hughlings Jackson Lectureship to commemorate "the Founder of the New Neurology," as Sir Thomas Barlow happily expressed his preeminence; their first President (1885) Hughlings Jackson was appointed the first lecturer, and so escaped the embarrassing experience

[2] Thayer, W. S.: *Bull. Johns Hopkins Hosp.*, Baltimore, 1927, xli, 10.

of listening, as would otherwise have been inevitable, to a lecture containing the expression of warm affection and intense admiration felt by his pupils and colleagues. Perhaps the same wise course was planned in this instance, but frustrated by William Sydney Thayer's true modesty which has very definitely dictated that in these two lectures there should not be any attempt at appreciation of his work, as I have known it since 1891, when we first met through a mutual friend A. A. Kanthack (1863–98), whose career of the highest promise was, to the great loss of British pathology, cut short after little more than a year's tenure of the Chair of Pathology at Cambridge. Much has changed since those far off days, but as the years have added up, my regard has only grown into increasing affection for Thayer. We in Old England regard him as another Osler whose tradition of unity, peace, and concord means so much to us, and even more to you as better able to judge of these two Masters of Medicine who have captured not only the minds but the hearts of their colleagues and pupils.

THE HEREDITARY FACTOR IN SOME DISEASES OF THE HAEMOPOIETIC SYSTEM

The new knowledge of heredity, which like statistics has now become too complicated for the plain medical man in the street, and the revival of interest in the constitutional factor in disease, which suffered an eclipse as the result of the rise of bacteriology, have coincided with an elaboration of the various forms of disorders of the haemopoietic system. New diseases of the blood-forming organs are constantly described based on histology, and in some instances on microchemical methods—data which provide information on the essentials of the hereditary and constitutional factors of disease. It may therefore not be out of place briefly to review our knowledge of the hereditary character of some morbid conditions of the haemopoietic system, an expression at once more accurate and inclusive than the old term "diseases of the blood." Such a consideration is of course attended by the underlying problem of heredity versus environment, for the less accentuated cases of hereditary influences may remain latent from lack of an exciting factor. Indeed, an extremely

interesting observation is the existence of latent manifestations in the blood and blood-forming organs, such as fragility, sickling of the red cells, and the persistence of haemoblastic foci in the liver in pernicious anaemia. These may not give rise to haemolytic anaemia until some additional factor, such as infection or toxaemia, intervenes. The practical distinction between hereditary and familial diseases may in some instances at least depend mainly on the age incidence and early mortality, for example, in grave familial jaundice of the newly born in which out of 130 collected cases 100, or 77 per cent, proved fatal. No doubt variations occur in the degree of the constitutional tendency, so that in some instances little in the way of an external environmental factor is needed to bring out the disease, whereas in others considerable intensity of the exciting factor is necessary.

Haemophilia

Haemophilia, first described by John C. Otto of Philadelphia in 1803, is the prototype and most characteristic of hereditary diseases, and is so well established that there is not any need for discussion. It is sex-limited and transmitted by females who do not suffer from the disease and are remarkably prolific. The transmission according to Nasse's law, or what Herringham called "Mother's son inheritance," without, as Bulloch and Fildes showed from analysis of 900 papers, any authentic female case, is very remarkable; for, as Nettleship pointed out in colour blindness and Leber's hereditary optic atrophy which are in like manner transmitted by unaffected females to males, females are occasionally attacked. It would be interesting to know if adrenal virilism in a female of a bleeder stock would prove an exception to Nasse's law. Haemophilia is imitated by hereditary pseudo-haemophilia and hereditary thrombopenia, in both of which females may be affected as well as males, and it has been suggested that purpura haemorrhagica is the female form of haemophilia (Hess[3]); the recognition of these and some other rare and somewhat obscure haemorrhagic conditions explains the difficulty in diagnosis when faced with what seems to be a female haemophiliac. As Giffin[4]

[3] Hess, A. F.: *Arch. Int. Med.*, Chicago, 1916, xvii, 203.
[4] Giffin, H. Z.: *Am. Journ. Med. Sc.*, Phila., 1928, clxxv, 44.

points out in recording a haemorrhagic disease, without the characteristics of haemophilia or purpura haemorrhagica, attacking women in four generations, there is much to be discovered about haemorrhagic diseases of this nature.

Chlorosis

Chlorosis is specially interesting as a disease, probably with an inborn tendency, in which the extrinsic or exciting factor has become rare. It has indeed been suggested that the universal decline in the incidence of this once familiar disease is more apparent than real, and due to the more frequent detection of cases of secondary anaemia, which before the era of focal infection and of blood counts as an almost routine practice were included under the caption of chlorosis (Aubertin and Mouquin); but at the best this would appear to be a very partial explanation, and perhaps naturally does not appeal to those who, only too familiar with the disease in the 'nineties of the last century, now look in vain for a case. Cabot twenty years ago excluded this explanation on the ground that there was then an actual diminution in the number of cases diagnosed as secondary anaemia without demonstrable cause. The striking diminution amounting almost to disappearance of chlorosis since the end of the last century in Europe and America has been ascribed to several factors, for example, to improved hygienic conditions, such as better ventilation, more open-air and exercise, to a diet containing more iron, to diminished alcoholism on the part of the parents (Schauman), the effect appearing some twenty years later, and with probably more justification to the passing of the once popular corset and tight lacing (Rosenbach; Weber), and thus to be a disease due to fashion.

The clinical impression that chlorosis is hereditary and familial was expressed by Potain and Clifford Allbutt, but statistics on this point do not appear to be available. Lloyd Jones' observations showed that the specific gravity of the blood in chlorotic females is low, and that this character is shared to a certain extent by their male relatives; he found that chlorotic families are remarkable for their fertility, and suggests that the condition of chlorotic blood, characterized by corpuscular defect, plasma of a normal specific gravity and excessive in quantity, is favourable to fertility, and indeed may be regarded

as an index of fertility of the mother and possibly of the facultative fertility of her offspring.

Erythraemia

Erythraemia, which has been shown by Christian to be accompanied by clinical manifestations curiously like those of pernicious anaemia and to resemble those of cerebral anoxaemia, usually occurs in persons between thirty-five and fifty years of age, and might therefore be thought to be more probably due to environmental influences than to constitutional factors. Weber (1921), after referring to cases recorded in early life, raised the possibility of a congenital incidence and the hypothesis that they may represent an extreme degree of a congenital plethoric tendency in some persons, but expressed his scepticism of their existence. Lately, however, its familial and constitutional incidence has attracted attention from Curschmann, Signorelli, and Trevor Owen; Engelking recorded the condition in three generations, grandmother, mother, and five children, and from the occurrence of infantilism in all the five children argued in favour of a defect in the germ plasm. Weber (1927) quotes familial and hereditary cases (Bernstein, Nichamin, Tancré, and Moewis), and now considers that in all kinds of primary and secondary polycythaemia a constitutional tendency—hereditary or familial—may play an important part; for example, in the presence of hyperpiesia or granular kidney this constitutional factor may convert the clinical picture into that of Geisböck's polycythaemia hypertonica.

Harrop and Heath, in a paper to which Professor W. T. Longcope has kindly drawn my attention, point out that lowered functional capacity of the lungs for gas diffusion may be an inherited character and occur in members of a family including an erythraemic patient, as is supported by the detection of apparently healthy relatives with a high red blood count and splenomegaly. From investigation of 7 cases they obtained evidence that there was diminution of the diffusion constant, and that disturbance of gas diffusion led to the compensatory erythraemia. This interference with pulmonary gas diffusion may be due to some structural abnormality in the pulmonary epithelium. The condition of erythraemia may remain latent for years and only become embarrassing with the advent of pulmonary em-

physema, fibrosis, high blood pressure and cerebral arteriosclerosis. An acquired condition, unique of its kind, of microscopic infiltration of the walls of the pulmonary alveoli by myosarcomatous metastases causing erythraemia (Barnes, Thompson, and Lamb) is analogous, though not homologous, to the constitutional defect in the pulmonary epithelium postulated by Harrop and Heath.

Sickle-celled blood

In 1904 Dresbach described an elliptical condition in 90 per cent of the red cells in a healthy mulatto; in 1910 J. B. Herrick gave an account of peculiar elongated red cells in a jaundiced negro with severe anaemia, leucocytosis and an ulcer of the leg. Cases by Washburn, Bishop who found two in a family, Mason, and others, were recorded as curiosities. In 1923 Huck showed that it was not rare as a latent condition, and from study of 11 persons in one family concluded that it conforms to the Mendelian law for the inheritance of a single factor and is dominant over the normal condition. In 1924 Sydenstricker observed 80 cases in 10 families, 71 being latent sicklaemia. Archibald recorded a case in an Arab boy in the Sudan. Cooley and Lee examined 400 negro children attending hospital, though not for anaemia, and found 30, or 7.5 per cent, with sickle-cells; they employed the word "sacklaemia" as a synonym for sicklaemia. Hahn and Gillespie suggested the words drepanocyte, from δρεπάνη = a sickle, for the sickle-cell, drepanocytaemia for the latent cases without symptoms, and drepanocytic anaemia for the active cases with anaemia, haemolytic jaundice and often ulceration of the legs and splenic enlargement; from investigation they found that the formation of sickle-cells is induced by anoxaemia, and that the spleen plays a secondary part, namely in excessive haemolysis of the sickle-cells, and is damaged in the process, passing through the stages of enlargement to subsequent atrophy. This may explain the percentage of 15 as the incidence of splenomegaly in the reported cases of sickle-celled anaemia (Hahn, 1928). In Hahn and Gillespie's case, which was at first regarded as chronic haemolytic jaundice although the resistance of the red cells to haemolysis by hypotonic salt solutions was recognised as inconsistent with this diagnosis, splenectomy was followed by great improvement in the blood count, but the condition

of sickling persisted; persistence of sickling was subsequently reported after splenectomy in two coloured children aged $1\frac{1}{2}$ and $2\frac{1}{2}$ years by Bell, Kotte, and Mitchell and another by Hahn (1928). These observations suggest that the spleen is not responsible for the sickle-cell trait; this is analogous to the persistence of fragility after splenectomy in chronic haemolytic jaundice.

The sickle-cell trait, which has been the subject of many papers in America, has been regarded as confined to negroes and as a hereditary anomaly of the red cells. It has, however, been recorded in a white Cuban boy without any direct negro ancestors (Stewart), in an Italian boy (Castana), and in 6 white adults by Lawrence. Possibly these exceptional cases belong to the at present small group isolated by Huck and Bigalow who collected 5 cases, including those previously reported by Dresbach and Bishop, in whites as well as blacks, of elliptical red cells in otherwise normal individuals. This condition is regarded by Huck and Bigalow as in no way related to the sickle-cell trait, for the abnormal cells are found in fresh blood and no change is induced by standing, as occurs in the sickle-celled form. The suggestion is thus made of an abnormal poikilocytosis which is distinct from sicklaemia. On the other hand Lawrence's observations tend to throw doubt on a sharp racial limitation of sickle-celled anaemia and on an absolute distinction between sickle or elliptical cells in whites and sickle-celled anaemia in negroes; in a white woman under his care with moderate anaemia and numerous sickle-cells he suggests that the condition may "represent a mild form of sickle-celled anaemia or some intermediary condition." Further, his results showed that in normal individuals of white and negro race there is very little difference in the incidence of the deformed cells, which were found in 2 per cent of negroes and in a slightly higher percentage of the whites examined.

It is interesting to compare sickle-celled anaemia with another hereditary blood anomaly, also regarded as possessing dominant Mendelian characteristics, namely, chronic haemolytic jaundice: both may be latent, though the increased fragility in chronic haemolytic jaundice has very rarely been reported in otherwise normal persons; nucleated reds in small numbers occur in chronic haemolytic jaundice and sickle-cells may be nucleated. The resistance of the reds to

hypotonic salt solutions, which is characteristically diminished in chronic haemolytic jaundice, is normal or even increased in sickle-celled blood.

Hahn and Gillespie figure a wet smear culture of blood, 48 hours after preparation, of sickle-celled anaemia showing "apparent colonization of the corpuscles around granular areas," a condition not previously reported. The figure suggests auto-agglutination, and my colleague Professor H. R. Dean tells me that though auto-agglutination is not constant in pernicious anaemia its presence is to his mind diagnostic.

Pernicious anaemia

Hereditary and constitutional influences have recently been raised as underlying factors which enable infection to excite the syndrome of pernicious (Addisonian) anaemia. The difficulties of getting a reliable history from hospital patients may in some degree account for the paucity in the past of reference to the heredo-familial factor; Cabot's analysis of 1200 collected cases in 1908 provided 2 examples only of its familial incidence, and Hurst (1927) never succeeded in obtaining a history of its family occurrence in hospital patients, though he and others have observed and collected many examples among private patients. The familial tendency appears to be more frequent than the hereditary; Mustelin's, Hurst's (1924), and Todd's records of the disease in three generations are rather exceptional; but there are many in two generations; B. Bramwell described 7 and Bartlett 8 cases in two generations; and in 1918 Ossian Schauman collected 24 families with a history of hereditary pernicious anaemia, either idiopathic or associated with tapeworm. Among 143 cases of pernicious anaemia in hospital and private practice, analysed by Levine and Ladd, there were 9, or 6.3 per cent, in which the disease occurred in relatives—parents, brothers, sisters, or children. It has been regarded as a dominant factor from a Mendelian point of view (Mustelin).

In contrast to sickle-celled anaemia, there is not any evidence of inherent defect in the red blood corpuscles preceding the onset of, or responsible for, the pernicious anaemia. But as achlorhydria is generally accepted as the early change in pernicious anaemia, Baumgartner and Hubbard's observations on the blood picture in achlor-

hydria may be quoted: among 153 persons on whom fractional gastric analyses were done, 40 were found to have pernicious anaemia, but among the remaining 113 there were only 16 with definite anaemia, 3 having less than 3,500,000 red cells. The increased resistance of the red cells in pernicious anaemia to hypotonic salt solutions contrasts with their fragility in chronic haemolytic jaundice. The achlorhydria, almost constant in pernicious anaemia (according to Hurst in 98.3 per cent), is thought to favour the incidence of anaemia by allowing the bacterial flora of the gastro-intestinal tract to grow in unrestrained profusion. Martius, in 1897, regarded achlorhydria as a true benign constitutional condition, and more recently it has been found in otherwise normal relatives of patients with pernicious anaemia; Hurst considers pernicious anaemia, sore tongue and subacute combined degeneration of the spinal cord as constitutional conditions, inasmuch as they depend on constitutional achlorhydria. Among healthy persons achlorhydria occurs in 4 per cent and among these pernicious anaemia will supervene in 16 per cent, and subacute combined degeneration of the cord in others. The question must then arise as to the degree of the immediate exciting cause—infection—that determines the anaemia, Hunterian glossitis or the spinal cord lesion. Hurst and Bell argue that together with a haemolysin a neurotoxin is produced in the alimentary canal by bacterial activity, and that according to the relative amounts of these two toxins anaemia or nervous manifestations are predominant. This explanation of the cord changes, which Woltmann found in 80.6 per cent of 150 cases of pernicious anaemia, is attractive, but has not escaped opposition; Piney argues from the absence of cicatricial (gliotic) tissue and reaction around the degenerations that they are not toxic but due to abiotrophy—a premature wearing out due to inherent defect in formation.

Knud Faber, while firmly convinced that pernicious anemia is due to an intestinal toxin, vigorously criticizes the view that achlorhydria is due to any constitutional or inborn defect, and brings forward much evidence to shew that achlorhydria is the result of gastritis; he admits that achlorhydria is the most frequent, but not the only, antecedent of pernicious anaemia, and in support of his contention refers to his report of 5 definite cases without achlorhydria. As

gastritis or gastro-enteritis, due either to direct damage or to toxins brought by the blood-stream, may produce temporary or long-continued achlorhydria in young children, the argument that its occurrence in early life is in favour of a constitutional origin is invalidated. It might be suggested that an inherent want of resistance to microbic invasion may play a part in the occurrence of pernicious anaemia, and that if achlorhydria, from whatever cause, occurs in such an individual, pernicious anaemia is more likely to ensue.

A different hypothesis bearing on the familial incidence of pernicious anaemia has been propounded by Piney; he finds constantly in the blood channels of the livers of these patients nests of pure megaloblastic tissue which can only produce megaloblasts, and argues that as the result of their abnormal persistence the red bone marrow fails in its function of producing normoblasts; thus he concludes that pernicious anaemia occurs only in those persons with this congenital malformation of a persistence of megaloblastic tissue in the liver. Oral or other sepsis, syphilis, and tapeworms in the intestine will in such disposed persons produce pernicious anaemia, but in persons with normal livers sepsis will lead to secondary anaemia only. In support of this argument it is significant that only a small proportion of persons with sepsis or tapeworm develop pernicious anaemia; in Scandinavia a large proportion of the inhabitants have tapeworm, in Finland one-third, and only a small proportion of these carriers, it is said 1 in 1,000 in Sweden, suffer from pernicious anaemia. Piney also rather tentatively suggests that the achlorhydria and subacute combined degeneration of the spinal cord seen in pernicious anaemia may be correlated phenomena, the cord changes being of the nature of an abiotrophy. The remarkable benefit that has followed the administration of liver substance (Minot and Murphy) or of a non-protein ether-insoluble, alcohol-precipitable, water-soluble fraction of beef protein (Minot, Murphy, Cohn, Stetson, and Lawson) to patients with pernicious anaemia and sprue and its apparently usual failure in secondary anaemia has been explained on the hypothesis that it stimulates the maturation of the megaloblasts which are present in large numbers in the bone marrow in pernicious anaemia, but not in secondary anaemia. Minot and Murphy also consider that the anaemia is primarily due to a defect in the formation of the red blood

cells and not to haemolysis. The difficult question arises if and to what extent this therapeutic effect of liver bears on the etiology of pernicious anaemia; does it lend support to Piney's view that one of the six conditions which he describes as coming under the syndrome of pernicious anaemia is due to a malformation of the liver; or is it compatible with the hypothesis that pernicious anaemia is of the nature of a deficiency disease, as might be suggested by the statement that the value of the treatment by liver substance depends on its content of vitamin E and iron (Simonds, Becker, and McCollom)? These are questions for future decision.

In connection with the question of a constitutional factor in pernicious anaemia, which may enable intestinal infection or tapeworm to precipitate the syndrome, the relation of sprue to pernicious anaemia is of considerable interest. These two conditions have recently been regarded as very closely allied (B. Bramwell); Wood (1925) suggests that "they may be types of a single condition," and accepts Ashford's view that sprue is due to infection by *Monilia psilosis*, this fungus having been isolated in all his recent cases of pernicious anaemia. It may be remembered that liver substance is an old remedy in Ceylon for sprue (Chalmers and Castellani). On the other hand, Baumgartner and Smith, who found achylia in 5 only and *Monilia* in 14 out of 15 cases of sprue, and out of 17 cases of pernicious anaemia 4 only with *Monilia*, dispute the identity of the two conditions, and the etiological bearing of yeast-like fungi on both sprue and pernicious anaemia has recently been denied by Nye, Zerfas and Cornwell. Christian, writing on "The Achlorhydria Family Tree of Disease," discusses their relationships and recognizes pernicious anaemia as a sequel of sprue, but points out that in sprue achlorhydria supervenes after the intestinal symptoms have been present for some time, whereas it precedes the blood changes of pernicious anaemia. It would therefore appear that sprue produces achlorhydria and thus paves the way for the development of pernicious anaemia. Thus achlorhydria, whether inherent and constitutional or acquired, would appear to be the antecedent to the blood picture of pernicious anaemia. But as only a certain proportion of the subjects of achlorhydria suffer from pernicious anaemia another factor is necessary; this may be the degree of intestinal toxaemia rendered possible, or there may be some in-

herent susceptibility to these toxins or, as Draper and Barach suggest, a change in the permeability of the intestinal wall or a peculiar genetic fault in the bone marrow.

Addison described the physique of patients with pernicious anaemia as chiefly of a somewhat large and bulky frame and with a strongly marked tendency to the formation of fat; G. Draper from anthropometric measurements finds that the chest is short, deep and has a very wide costal margin, the profile characterized by a very wide jaw angle, a massive lower jaw in proportion to the other facial measurements, wide face with very great interpupillary space (Draper, Dunn, and Seegal), and that males have the longest upper extremities of any disease pattern. Writing with Barach, he has come to the conclusion that there is a psychical as well as a physical pattern in the pernicious anaemia race which represents an approach to the neuter or species type characterized by an arrest of differentiation in the psyche and the stroma; this hypothesis will no doubt be stated more fully after further investigation. Levine and Ladd's figures show that the disease is rare in Jews and in Russians, and less often seen in the dark-haired than in the light-haired.

Chronic haemolytic jaundice

Chronic haemolytic jaundice may be (1) acquired or (2) hereditary, congenital, and familial. With regard to the acquired form which presents resemblances to pernicious anaemia the question arises whether or not it depends solely on infection or whether there is not an underlying, though perhaps slight, constitutional liability, without which the infection would not produce this effect. The hereditary form must start from an accentuation of latent tendencies in the parents, and the first case may appear to be acquired, so that subsequently the condition shews the phenomenon of anticipation and becomes familial and congenital. It would thus seem reasonable to regard the two forms usually described as the acquired and the hereditary as both dependent on a constitutional factor, though this is of different degree of intensity.

Hereditary, congenital, and familial haemolytic jaundice

In 1887 Spencer Wells removed from a woman aged 24 years the spleen which before operation was thought to be a uterine or ovarian tumour; like his operation on what was diagnosed as an ovarian cyst but turned out to be tuberculous peritonitis, the abdomen being closed with a depressing prognosis, and a happy recovery, this was an example of what may be called a fortunate mistake. Since the age of 9 years this patient had been subject to recurring attacks of jaundice, which ceased after splenectomy. In 1914 she was well, but her red blood corpuscles still shewed fragility (Dawson). This observation is important as bearing on the question whether the fragility of the reds is an hereditary character or whether it is due to the influence of the spleen. Although it has been regarded as exceptional (Moynihan), fragility of the red cells appears to persist after splenectomy (Campbell and Warner), and hence this blood trait is not dependent on the spleen and may be regarded as a constitutional anomaly. In a few recorded cases (Giffen and Sanford; Campbell and Warner) relatives of patients with chronic haemolytic jaundice have shewn the characteristic fragility of the red blood corpuscles and, though apparently normal, may convey that disease to their children. Further, according to Campbell and Warner the disease behaves as a Mendelian dominant.

The small size of the red cells, first noticed by Chauffard, was thought by Naegeli to indicate that the patients belonged to a different type of the human species; but against this interesting conception there are two points: in the first place, Meulengracht's statement that it is confined to those members of the family with symptoms and not found, as fragility has been, in their otherwise normal relatives, and secondly, that according to Whitcher the microcytosis tends to pass away after splenectomy and so is presumably due to the presence of the spleen. In regard to the effect of splenectomy it might be considered that this is not an argument of value, as this operation might interfere with the congenital anomalous mechanism responsible for the microcytosis. The number of records on the effect of splenectomy on the fragility and especially on the microcytosis is small, and further observations are desirable on the questions whether the persistence of the fragility is absolute and exactly the same as before splenectomy,

and whether the microcytosis diminishes or completely disappears. Piney, in a review of the subject, regards the excessive fragility and microcytosis as secondary phenomena, and is inclined to suggest merely an inherent susceptibility on which an additional external factor is able to produce these phenomena, and not to accept as an inherited morbid process the fragility. It is, however, tempting to anticipate further evidence to the effect that the fragility, though not the microcytosis, is an inherited anomaly and may be latent until some external factor, such as infection, stimulates the spleen to greater haemolytic activity so that anaemia and jaundice result. The analogy of sickle-celled anaemia, which is so often latent, makes this view attractive.

It is interesting to compare the conditions in pernicious anaemia, on the one hand, with those in chronic haemolytic anaemia and the sickle-celled trait and anaemia in negroes on the other hand. In pernicious anaemia there is little if anything in the way of a latent blood change, such as is present in latent cases of the sickle-cell anaemia in negroes, and, though this is not so widely established, the increased fragility in the relatives of the subjects with chronic haemolytic jaundice. Achlorhydria is almost constant in pernicious anaemia, but in the other two little is known in this respect. Professor Longcope has kindly given me the details of two cases of sickle-celled anaemia in the Johns Hopkins Hospital, which suggest that these patients are able to secrete HCl, although gastric analysis may sometimes fail to shew its presence. It might be urged that in pernicious anaemia, which is often regarded as a syndrome rather than a disease, the constitutional factor is mainly in the direction of favouring, by the existence of achlorhydria, the occurrence of the directly exciting factor, bacterial infection; whereas in chronic haemolytic jaundice and sickle-celled anaemia there is an inborn defect in the red blood corpuscles which readily undergo haemolysis when any extrinsic factor comes on the scene.

Gaucher's disease and Niemann's disease

These diseases attacking the reticulo-endothelial system, which are referred to in connection with their incidence in Jews in the second lecture, have been separated from the cases of splenomegaly called

chronic splenic anaemia. Quite recently Niemann's disease, of which few cases are as yet on record, has been differentiated from Gaucher's disease on the grounds of their micro-chemical and clinical characters, though Graham and Blacklock have contested the sharp differentiation based on the micro-chemical reactions of the material in the large cells in the two conditions.

Gaucher's disease (large-celled splenomegaly) was thoroughly investigated by the late F. S. Mandlebaum and his colleagues from 1904 to 1919. In 1926 Pick accepted 39 cases, Cushing and Stout collected 49 examples, and in 1927 Klercker brought the number up to 60. There is a distinct familial tendency, but the disease has not been shewn to occur in two generations. It has been stated to be familial in 40 per cent of the cases, and has been recorded in four members of a generation (Brill, Mandlebaum, and Libman). The familial incidence has been specially noted in cases with predominant involvement of the bones. Pick regards it as "a congenital, familial, constitutional anomaly of metabolism akin to alcaptonuria or cystinuria," and Waugh and MacIntosh argue that it is a congenital progressive systemic disease of the haemopoietic tissues, characterized by an aleukaemic dysmyelosis, *i.e.* an irregular perverse myeloid metaplasia, due to the same causes as leukaemia, and that it may be a constitutional anomaly, namely a mutation of the human species.

Niemann's disease, described in 1914 as a nutritional disease of infants of unknown nature, has been specially investigated by Pick who called it lipoid-celled splenohepatomegaly. Previously some cases in very early life had been recorded as Gaucher's disease. It contrasts with Gaucher's disease in its rapid and fatal termination and in the difference of the lipoid content of the large cells and the complex lipoprotein (kerasin) in the Gaucher cells. Among the few recorded cases it has been found in sisters (Knox, Wahl and Schmeisser; Siegmund), and in association with amaurotic family idiocy (Hamburger) and mongolism (Niemann; Bloom).

Infantile anaemia. (*Synonyms: von Jaksch's anaemia infantum pseudo-leukaemica, splenomegaly with anaemia and myelaemia*)

Under this heading several conditions, especially of secondary anaemia, have from time to time been included, so that the name has

become that of a syndrome rather than of a disease, and has thus been in much the same nosological position as chronic splenic anaemia of adults. But in at least one not very uncommon form the familial incidence and its occurrence in twins, of which John Thomson saw more than a dozen examples, have naturally suggested that there is an underlying hereditary anomaly (Marquard) which enables defective dietary or infection to cause anaemia the more readily and of a special kind. As an indication that there may be other forms of hereditary anaemia reference may be made to oligochromaemia porphyrinurica (megalosplenica congenita) described by Sato and Takahashi as a familial condition characterized by splenomegaly from birth, chlorotic anaemia, porphyrinuria, purple teeth, and a cutaneous eruption of the same nature as in congenital porphyrinuria.

Hodgkin's lymphogranuloma or lymphadenoma

Hodgkin's lymphogranuloma or lymphadenoma has in rare instances been known to attack two in the same family or twins (Senator; Peacock), thus suggesting that a special tissue susceptibility or constitution, such as status lymphaticus (Arkin), favours its incidence. As in the case of acute leukaemia which has been recorded in a nurse after contact with a case (Obrastzow) and in a brother and sister (Eppinger; Schereschewsky), and in which the question of hereditary and familial disposition has been considered by Bauer and Weber, the explanation that infection is responsible must arise; until the causal agents of these diseases are established it is impossible to deny that, as in the case of malignant disease, some constitutional factor may play a part in their incidence. But the cases clinically supporting the contention of a heredo-familial incidence are so rare that the evidence is not so definite as in the case of the tuberculous diathesis or the heredity of malignant disease.

REFERENCES

Chlorosis

ALLBUTT, CLIFFORD: System of Medicine (Allbutt), 1898, v, 483.

AUBERTIN ET MONQUIN: Nouveau traité de médecine (Roger, Widal et Teissier), Fasc. ix, p. 4, 1927.

CABOT, R. C.: Modern Medicine (Osler and McCrae), 1908, iv, 640.

JONES, E. LLOYD: Chlorosis, 1897, London.
ROSENBACH, O.: Corset und Bleichsucht, Stuttgart, 1895.
SCHAUMAN: Quoted in *Brit. Med. Journ.*, 1922, i, Epitome, No. 301.
WEBER, F. P.: *Brit. Med. Journ.*, 1925, i, 960.

Erythraemia

BARNES, THOMSON, AND LAMB: *Quart. Journ. Med.*, Oxford, 1925–6, xix, 151.
CHRISTIAN, H. A.: *Med. Clinics North America*, 1925, vi, 840.
CURSCHMANN, H.: *Acta Med. Scandinav.*, Stockholm, 1922, lviii, 228.
ENGELKING, E.: *Klin. Monatsbl. f. Augenheilk.*, 1920, lxiv, 645.
HARROP, G. A., JR., AND HEATH, E. H.: *Journ. Clin. Investig.*, 1927, iv, 53–70.
OWEN, T.: *Bull. Johns Hopkins Hosp.*, Baltimore, 1924, xxxv, 258.
SIGNORELLI, E.: *Haematologia*, 1923, iv, 437.
WEBER, F. P.: Polycythaemia, Erythrocytosis and Erythraemia, p. 54, 1921.
Idem: Med. Press and Circ., 1927, clxxv, 128.

Sickle-celled blood

ARCHIBALD, R. G.: *Trans. Roy. Soc. Trop. Med.*, 1926, xix, 389.
BELL, A. J., KOTTE, R. H., AND MITCHELL, A. G.: *Am. Journ. Dis. Child.*, Chicago, 1927, xxxiv, 923.
BISHOP, F. W.: *Arch. Int. Med.*, Chicago, 1914, xiv, 388.
CASTANA, V.: *Pediatria*, Napoli, 1925, xxxiii, 431.
COOLEY, T. B., AND LEE, P.: *Am. Journ. Dis. Child.*, 1926, xxxii, 334.
DRESBACH, M.: *Science*, 1904, N. S., xix, 469.
HAHN, E. V.: *Am. Journ. Med. Sc.*, Phila., 1928, clxxv, 206.
HAHN, E. V., AND GILLESPIE, E. B.: *Arch. Int. Med.*, Chicago, 1927, xxxix, 233.
HERRICK, J. B.: *Arch. Int. Med.*, Chicago, 1910, vi, 517.
HUCK, J. G.: *Bull. Johns Hopkins Hosp.*, Baltimore, 1923, xxxiv, 335.
HUCK, J. G., AND BIGALOW, R. M.: *Bull. Johns Hopkins Hosp.*, Baltimore, 1923, xxxiv, 390.
LAWRENCE, J. S.: *Journ. Clin. Investig.*, Baltimore, 1927, v, 31.
MASON, V. R.: *Journ. Am. Med. Assoc.*, Chicago, **79**, 1318, 1922.
STEWART, W. B.: *Am. Journ. Dis. Child.*, 1927, xxxiv, 72.
SYDENSTRICKER, V. P.: *Journ. Am. Med. Assoc.*, Chicago, **83**, 12, 1924.
WASHBURN, R. E.: *Virginia Med. Semi-Month.*, 1911, xv, 490.

Pernicious anaemia

ADDISON, T.: On the Constitutional and Local Effects of Disease of the Suprarenal Capsules, p. 3, 1854.
BARTLETT, J. C.: *Journ. Am. Med. Assoc.*, Chicago, **60**, 176, 1913.
BAUMGARTNER, E. A., AND HUBBARD, R. S.: *Clifton Med. Bull.*, 1927, xiii, 71.
BAUMGARTNER, E. A., AND SMITH, G. D.: *Arch. Int. Med.*, Chicago, 1927, xl, 203.
BRAMWELL, B.: Anaemia and Some Diseases of the Blood-forming Organs, 1899.

Idem: Brit. Med. Journ., 1924, i, 365.
CABOT, R. C.: Modern Medicine (Osler and McCrae), 1908, iv, 612.
CHALMERS AND CASTELLANI: Tropical Diseases, p. 1791, 1919.
CHRISTIAN, H. A.: *North-West Med.*, 1925, xxiv, 531.
DORST, S. E.: *Am. Journ. Med. Sc.*, Phila., clxxii, 173.
DRAPER, G.: Human Constitution, 1924.
DRAPER, G., AND BARACH, A. L.: *Journ. Clin. Investig.*, Baltimore, 1927, iv, 507.
DRAPER, G., DUNN, H. L., AND SEEGAL, D.: *Am. Journ. Med. Sc.*, Phila., 1925, clxix, 322
FABER, K.: Lectures on Internal Medicine, pp. 41, 72, 1927.
HURST, A. F.: Medical Essays and Addresses, p. 89, 1924.
Idem: Brit. Med. Journ., 1927, i, 825
HURST, A. F., AND BELL, J. R.: *Brain*, 1922, xlv, 266.
LEVINE, S. A., AND LADD, W. S.: *Bull. Johns Hopkins Hosp.*, Baltimore, 1921, xxxii, 254.
MARTIUS, F.: Achylia Gastrica, Leipzig, 1897.
MINOT, G. R., AND MURPHY, W. P.: *Brit. Med. Journ.*, 1927, ii, 674.
MINOT, MURPHY, COHN, STETSON, AND LAWSON: *Trans. Assoc. Am. Phys.*, Phila., 1927, xlii, 83.
MUSTELIN, O.: *Acta med. Scandinav.*, Stockholm, 1922, lvi, 411.
NYE, R. N., ZERFAS, L. G., AND CORNWELL, M. A.: *Am. Journ. Med. Sc.*, Phila., 1928, clxxv, 153.
PINEY, A.: Recent Advances in Haematology, 1927.
Idem: Proc. Roy. Soc. Med., 1925, xviii (Path. Sect.), 5.
SCHAUMAN, O.: Quoted by Meulengracht, E., *Am. Journ. Med. Sc.*, Phila., 1925, clxix, 177.
SIMMONDS, BECKER, AND MCCOLLOM: *Journ. Am. Med. Assoc.*, Chicago, **83**, 1047, 1927.
TODD, A. T.: *Brit. Med. Journ.*, 1926, ii, 180
WOLTMANN: Collected Papers of the Mayo Clinic, 1918, x, 933.
WOOD, E. J.: *Am. Journ. Med. Sc.*, Phila., 1925, clix, 28; Modern Medicine (Osler, McCrae, and Funk), 1926, iii, 649.

Chronic haemolytic jaundice

CAMPBELL, J. M. H., AND WARNER, E. C.: *Quart. Journ. Med.*, Oxford, 1925–26, xix, 333.
DAWSON, B.: *Proc. Roy. Soc. Med.*, 1914, vii (Clin. Sect.), 86.
GIFFIN AND SANDFORD: *Journ. Lab. and Clin. Med.*, 1919, iv, 465.
MOYNIHAN, B.: The Spleen, p. 98, Bristol, 1921.
NAEGELI, O.: Blutkrankheiten und Blutdiagnostik, 4th edition, 1923.
PINEY, A.: Recent Advances in Haematology, p. 240, 1927.
WELLS, T. SPENCER: *Med.-Chir. Trans.*, 1888, liii, 255.
WHITCHER, B. R.: *Am. Journ. Med. Sc.*, Phila., 1925, clxx, 678.

Gaucher's disease

BRILL, MANDLEBAUM, AND LIBMAN: *Am. Journ. Med. Sc.*, Phila., 1909, cxxxvii, 849.
CUSHING, E. H., AND STOUT, A. F.: *Arch. Surg.*, Chicago, 1926, xii, 539.
GRAHAM, S., AND BLACKLOCK, J. W. S.: *Arch. Dis. Child.*, London, 1927, ii, 267.
KLERCKER, KJ. O. AF.: *Acta Paediat.*, Stockholm, 1927, vi, 302.
MANDLEBAUM, F. S.: *Am. Journ. Med. Sc.*, Phila., 1919, clvii, 366.
PICK, L.: *Ergebn. der inn. Med. u. Kinderh.*, Berlin, 1926, xxix, 519.
WAUGH, T. R., AND MACINTOSH, D. S.: *Arch. Int. Med.*, Chicago, 1924, xxxiii, 599.

Niemann's disease

BLOOM, W.: *Am. Journ. Path.*, Boston, 1925, i, 595.
HAMBURGER, R.: *Jahrb. f. Kinderheilk.*, 1927, cxvi, 41.
KNOX, WAHL, AND SCHMEISSER: *Bull. Johns Hopkins Hosp.*, Baltimore, 1916, xxvii, 109.
NIEMANN, A.: *Jahrb. f. Kinderheilk.*, 1914, lxxix, 1.

Infantile anaemia

MARQUARD, E.: *Arch. f. Kinderheilk.*, 1923, lxxii, 251.
SATO, A., AND TAKAHASHI, H.: *Am. Journ. Dis. Child.*, 1926, xxxiii, 325.
THOMSON, J.: Clinical Study and Treatment of Sick Children, p. 425, 1925.

Hodgkin's lymphogranuloma

ARKIN, A.: *Am. Journ. Med. Sc.*, Phila., 1926, clxx, 669.
BAUER, J.: Die konstitutionelle Disposition zu inneren Krankheiten, 1924, p. 257.
OBRASTZOW: *Deutsche med. Wchnschr.*, 1890, xvi, 1130.
PEACOCK, G.: *Dublin Journ. Med. Sc.*, 1905, cxx, 85.
SCHERESCHEWSKY, E.: *Zntrlbl. f. d. inn. Med.*, 1926, xlvii, 643.
SENATOR, H.: *Deutsche Klinik*, 1903, iii, 373.
WEBER, F. P.: *Med. Press and Circ.*, 1927, clxxv, 128.

ELECTROGRAMS TAKEN FROM ISOLATED STRIPS OF MAMMALIAN VENTRICULAR CARDIAC MUSCLE[1]

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It has been shown in a previous paper (1) that it is possible to obtain rhythmic contractions from isolated muscle strips of the mammalian ventricle suspended in oxygenated Ringer's solution. The following brief report is concerned with the electrograms obtained from similar rhythmically contracting muscle strips.

The technique followed in this study was essentially that reported in the former paper. The muscle strips were suspended in oxygenated Ringer's solution made with triply distilled water and pure chemicals (Kahlbaum). The addition of defibrinated blood was found unnecessary, but adrenalin was added as needed. The electrical tracings were taken with non-polarizable electrodes made of kaolin paste and copper sulphate placed close to, but not touching, the muscle. The strip was suspended horizontally in a shallow dish with a recording lever attached at right angles. This apparatus was set up directly in front of the film of the string galvanometer in such a way that the mechanical record could be photographed upon the same film as the electrical record.

The curves were taken from rhythmically contracting strips of ventricular muscle. These showed considerable variation in rate, rhythm, and in the form of the electrical complexes. The rate varied from 20 per minute, or even slower at the initiation of rhythmicity, up to 176 per minute. This, however, does not necessarily mark either the upper or the lower limit of rate observable in isolated muscle strips. While records were being taken, the temperature of the immersion fluid was kept constant within four degrees—the maximum being 34°C.; the minimum 30°C.

[1] Received for publication, April 24, 1928.

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Examples of the more significant tracings together with the protocols of the experiments are as follows:

Expt. 3. Cat anaesthetized with ether at 11.05 a.m. 11.20, Heart excised. 11.27, Papillary muscle strip (1.3 cm. long, 0.5 cm. wide) immersed in oxygenated Ringer's solution pH 7.8. Temperature 31°C. 18 minutes later one drop of adrenalin (1:1000 dilution) was added to the immersion fluid—Temp. 34°C.—and the record shown in Figure 1 was taken. This record is of special interest because it shows definite alternation in the recovery phase.

Expt. 4. Cat anaesthetized at 9.50 a.m. (A strip (1.5 cm. x 0.5 cm.) taken from the posterior papillary muscle of the left ventricle was immersed in oxygenated Ringer's solution pH 7.8 at 10.15 a.m. The record shown in Figure 2 was taken one hour later with direct leads of fine silver wire piercing the muscle strip. This record shows a recovery phase of great amplitude and of long duration. The technique of direct leads was subsequently given up owing to the

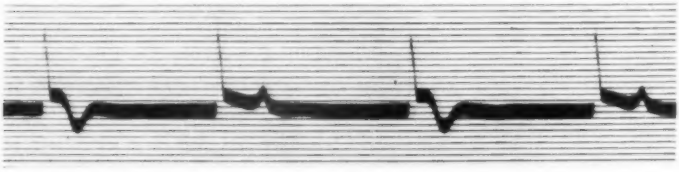


FIG. 1. EXPT. 3

Strip from posterior papillary muscle of cat heart shows alternation in the recovery phase. String standardization, 2 cm. = 1 millivolt.

possibility that the rhythmic response might be due to muscle injury at the points of contact and also because of the possibility of polarization of the metal electrodes.

Expt. 15. Cat anaesthetized with ether at 11.17 a.m. 11.30, heart excised. 11.35 a.m. strip of the posterior papillary muscle (1 cm. long, 0.3 cm. wide, and 0.2 cm. thick) immersed in oxygenated Ringer's solution pH 8.4. Temperature 32°C. The electrodes were placed widely apart, one at one end, the other nearly, but not quite, at the opposite end of the muscle strip. There was considerable difficulty in adjusting the mechanical writing lever and the electrodes. Rhythmic contractions continued for a short time and then ceased. At 1.47 p.m. two drops of adrenalin (1:1000) were added to the Ringer's solution; at 2.20 p.m. two more drops of adrenalin (1:1000) were added and three minutes later record 7 (Fig. 3) was taken. Here the dominant rhythm is interrupted at irregular intervals by a beat whose deflection is in the opposite direction. Inasmuch as the electrodes were not changed while the record was being taken, the presumption is that these represent beats arising from another focus at the

opposite end of the strip. It will also be noted that during the dominant rhythm the recovery phase is more distinctly visible here than in the other records with indirect leads. At 3.15 p.m. adrenalin (2 drops of 1:1000 dilution) was again added to the immersion fluid. Immediately thereafter records 11 to 13 (Fig. 4)

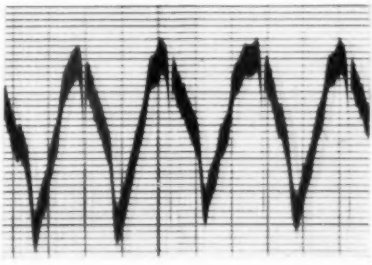


FIG. 2. EXPT. 4

Tracing taken from posterior papillary muscle of cat heart with direct leads. String standardization, 2 cm. = 1 millivolt.

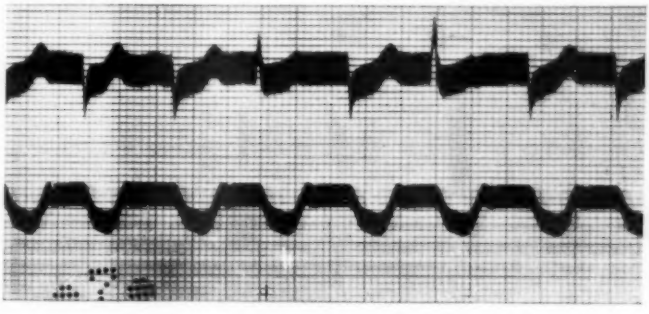


FIG 3. EXPT. 15

Record 7 from posterior papillary muscle of cat heart showing a rhythm interrupted occasionally by beats arising at the opposite end of the muscle strip. The electrical response is shown by the upper tracing; the mechanical, by the the lower. String standardization, 3 cm. = 1 millivolt.

were taken and two minutes later, at 3.17, record 14 (also shown in in Fig. 4). Record 11 might be interpreted as a re-entrant rhythm. The string is in almost constant motion. The rate is rapid—approximately 143 a minute, but not absolutely regular. In record 12 the rate is slightly slower—130 a minute—and

there is a suggestion of an iso-electric period. This is seen slightly more clearly in record 13 in which the rate is still slower—125 a minute, whereas in record 14, taken but 2 minutes after record 11, the rate has markedly slowed. It is but 38 per minute and the form of electrical complex remains essentially the same and distinct iso-electric periods are seen between the beats. In this curve there can be no question; it clearly does not represent a re-entrant rhythm.

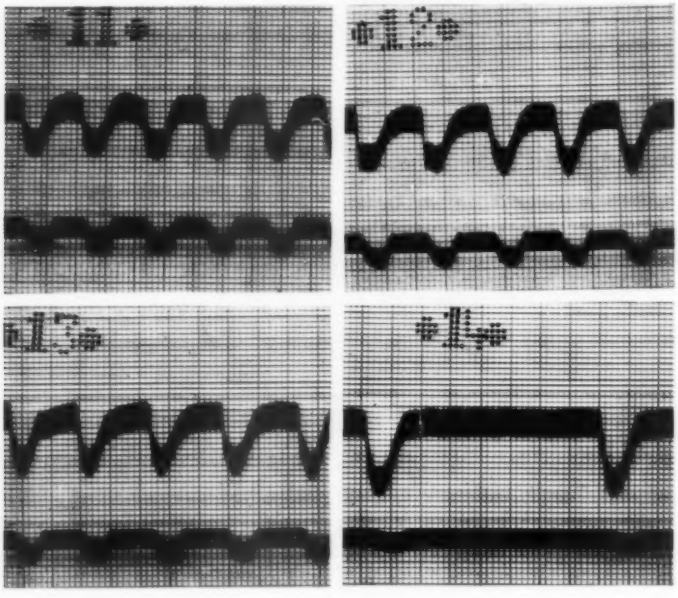


FIG. 4. EXPT. 15

Records 11 to 14. Tracings taken from posterior papillary muscle of cat heart immediately after the addition of adrenalin. The upper tracing shows the electrical response; the lower, the mechanical. String standardization, 3 cm. = 1 millivolt.

At 4 p.m. the oxygen was turned off. It was turned on again at 4.40 p.m. and 2 drops of adrenalin (1:1000) were added and records 20 and 21 (Fig. 5) were taken. The curves show that the strip still possessed rhythmicity for more than five hours after the experiment was started. Furthermore, in these records the electrical phenomena were readily recorded although the mechanical excursions were very weak. Immediately after this record had been taken the experiment was closed.

Expt. 17. 10.01 a.m. Cat anaesthetized with ether. 10.10, heart excised. 10.15 a.m., a strip (2 cm. long, 0.4 cm. broad and 0.2 cm. thick), taken from external layer of left ventricle, was immersed in oxygenated Ringer's solution pH 7.9—Temp. 32°C. Between 10.15 a.m. and 11 a.m. several pairs of kaolin electrodes were made in an effort to increase their sensitivity. At 10.30 a.m. adrenalin (3 drops of 1:1000 dilution) was added to the Ringer's solution. At 10.38 a.m., although the strip was beating, three more drops of adrenalin were added in an attempt to increase the force of contractions. Between 11.05 a.m. and 11.15 a m. records 8 to 12 (Fig. 6) were taken in rapid succession with the same pair of electrodes under similar conditions, *i.e.*, the temperature was constant at 31.5°C. and there was no change made in mechanical adjustments. The

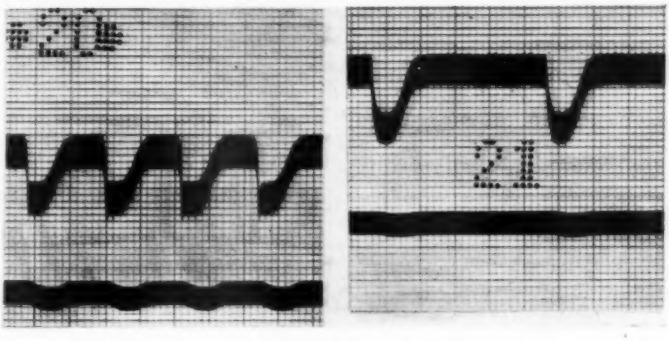


FIG. 5. EXPT. 15

Records 20 to 21 showing response after the addition of adrenalin over 5 hours after the strip was placed in Ringer's solution. The electrical response is shown by the upper tracing; the mechanical, by the lower. String standardization, 3 cm. = 1 millivolt.

only difference in the taking of these records was in the position of the electrodes. The difference in the resulting curves is striking. At 11.05 a.m. record 8 was taken with electrodes placed one at either end of the strip. The electrical deflections were very small and difficult to make out. In record 9, taken six minutes later (11.11 a.m.), the electrodes had been shifted so that they were close together near the center of the strip. Oxygen was bubbling through the solution continuously. In order to eliminate any artefact due to the effect of oxygen, it was turned off, and record 10 was taken immediately thereafter (11.12 a.m.). In record 11, taken two minutes later with oxygen bubbling through the solution, the position of the electrodes was unchanged. In record 12 the oxygen was again shut off. The amplitude of the deflections is less, particularly the end phase, but otherwise the form of the complexes remains essentially the same; in both,

the initial and end phases are diphasic. The significance of the form of these curves will be discussed later.

In records 21 to 24 (Fig. 7) the effect of shifting the position of the electrodes was again tested. These records were taken in rapid succession at 11.30 a.m. Temp. 30.5°C. The oxygen was shut off while the first three records were taken.

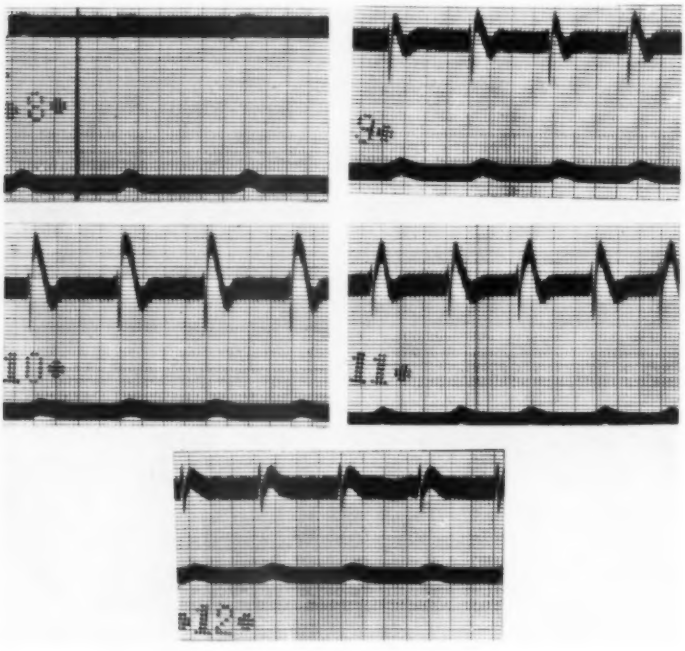


FIG. 6. EXPT. 17

Records 8 to 12, taken from a strip of external layer of left ventricle of cat heart, illustrate the relation of the position of the electrodes to the form of the electrical complex. The electrical tracing is the upper; the mechanical, the lower. String standardization, 2 cm. = 1 millivolt.

No adrenalin had been added since 10.38 a.m. In record 21, the electrodes were about 0.5 cm. apart and both were nearer one end of the muscle strip than the other. The recorded curve is of low potential. In record 22 the electrodes were kept the same distance apart but shifted towards the center of the strip. The amplitude of the string deflection is markedly increased. It is distinctly monophasic in type and a small recovery phase is visible. In record 23 the elec-

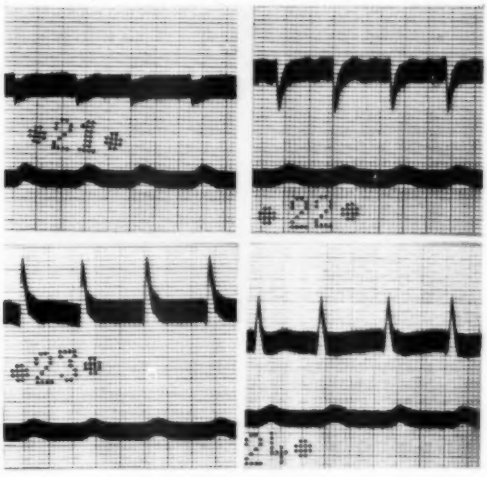


FIG. 7. EXPT. 17

Records 21 to 24. Tracing taken from external layer of left ventricle of cat heart. Again illustrates the change in the form of the electrical complex with change in the position of the electrodes. The electrical curve is the upper; the mechanical, the lower. String standardization, 2 cm. = 1 millivolt.

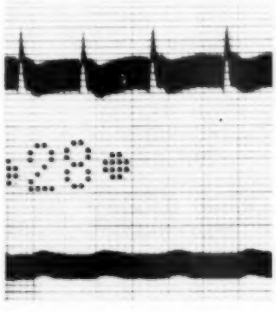


FIG. 8

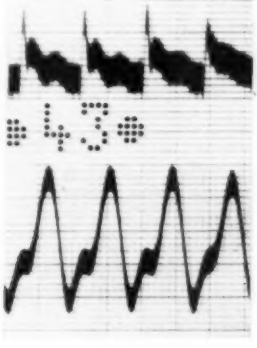


FIG. 9

FIG. 8. EXPT. 17

Record 28. Illustrates the effect of adrenalin upon a muscle strip taken from the external layer of the left ventricle of the cat heart. The electrical curve is the upper; the mechanical, the lower. String standardization, 2 cm. = 1 millivolt.

FIG. 9. EXPT. 17

Record 43. Shows a tracing taken 14 minutes after the last addition of adrenalin and 5½ hours after the muscle strip was excised from the heart. The electrical tracing is the upper; the mechanical, the lower. Oxygen running continuously. String standardization, 2 cm. = 1 millivolt.

trodes were reversed and, as was to be expected, the deflection was in the opposite direction. Before the last record of the series (No. 24) was taken it became necessary to turn on the oxygen, as the rate had fallen from 120 to 96 per minute and the rhythm was becoming distinctly more irregular. In this record the electrodes were spread widely apart, almost at either end of the strip. In this instance the variation in the resulting electrical complex is not striking.

At 11.42 a.m. adrenalin (3 drops of 1:1000 dil.) was added. The electrodes were distinctly separated but not placed at the extreme ends of the muscle strip. Record 28 (Fig. 8) was taken to show the effect of adrenalin. The rate is much more rapid, 150 a minute. The curve is triphasic in type.

Adrenalin was again added at 12.35 and 3.16 p.m. and 4.32 p.m. At 4.46 p.m., adrenalin having been added 14 minutes previously, record 43 (Fig. 9) was taken. It will be noted that the mechanical leverage has been greatly improved. The curve is complicated by the fact that the oxygen was bubbling through the solution, but nevertheless is of interest in that it shows not only rhythmicity but a rapid rate, 176 per minute at a temperature of 31°C., five and one-half hours after the strip was immersed in oxygenated Ringer's solution. The strip was still beating when the experiment was closed at 4.50 p.m.

DISCUSSION

Experiment 17 was planned in an attempt to determine whether the nature of the rhythm was the same in all cardiac muscle strips or whether that encountered in strips of pure cardiac tissue, i.e., free from specialized tissue, was due to re-excitation rather than to true inherent rhythmicity. For this reason the strip was cut from the external layer at the base of the left ventricle near the anterior longitudinal sulcus—thus taking tissue as far away as possible from the Purkinje system. Serial sections were made of the muscle strips used in both experiments 15 and 17. In each of these series alternate sections were stained with a hematoxylin and eosin stain and with an iron-hematoxylin stain. From the gross appearance alone we feel quite certain that the strip of papillary muscle (Expt. 15) did contain some Purkinje tissue. The strip taken from the external layer (Expt. 17) presented a more difficult problem. In such tissue one must differentiate between the less typical Purkinje cells, transitional cells, and true cardiac muscle cells. In the cat's heart the difference between these various types of cells is very slight and at present there is no method of differential staining. Therefore, it proved to be quite impossible to determine satisfactorily whether or not there was any specialized tissue in this strip.

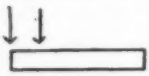
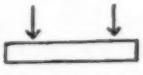
It is, however, apparent that both strips possessed the inherent power of true rhythmicity as shown by the definite iso-electric periods between the beats. If, however, the external layer does contain Purkinje cells, this system is very much more widely distributed throughout the ventricle than has been generally assumed.

These tracings show that the curves obtained from isolated strips of mammalian ventricular cardiac muscle are essentially of the same nature as those obtained experimentally from the intact heart. A number of tracings show an initial phase followed by a recovery phase of longer duration, and definite iso-electric periods between the beats. These curves also show a number of variations seen in the intact hearts—alternation, in this instance seen in the recovery phase, and extrasystoles arising from a secondary focus (Fig. 3).

Furthermore, these curves are of interest in the light of the hypothesis recently advanced by Craib (2). He demonstrated that the form of the electrical complex varies with the position of the electrodes. Thus, in experiment 15, record 7 (Fig. 3) the electrodes were placed one at the end, and the other nearly, but not quite at the opposite end,

and the curve is practically identical with the predicted curve. In records 11 to 14 (Fig. 4) which were taken one hour later, the curves are monophasic but their form is altered, though the position of the electrodes has not been changed. It is, however, quite possible that a new rhythm of increased rate was initiated by the adrenalin given just before these records were taken.

In experiment 17, the difference between the deflections recorded in records 8 and 9 (Fig. 6) is distinct. In this instance a shift in the position of the electrodes from the extremities to the center of the strip increased the recorded potential difference from an amplitude of less than 1 mm. to one of almost 19 mm. where 1 cm = 2 millivolts. This may or may not be of significance in that it is conceivable that the electrodes, when record 8 was taken, were not in proper contact with the ends of the muscle strip. Moving them slightly toward the center of the strip gave an initial deflection and a conspicuous end phase very similar in outline to the predicted curve with the electrodes at the center and end of the strip.

Again, in records 21 to 24 (Fig. 7) the effect of shifting the position of the electrodes was tested. In record 21, the electrodes were about 1 cm. apart and both nearer to one end of the muscle strip than the other  . The recorded curve, though of low potential, is not exactly that of the predicted curve. In record 22, the electrodes were kept the same distance apart as they were in record 21, but both were shifted towards the center of the strip. The amplitude of the string deflection is markedly increased but the form is not fundamentally changed. In record 23, the position of the electrodes is unchanged but the polarity reversed and, as previously noted, the deflection is in the opposite direction. In record 24, the electrodes were spread widely apart, almost at either end of the strip. This curve is essentially that of the predicted curve. The variation in the complexes between this record and the preceding three records is less striking than one might expect. Nevertheless, it must be remembered that the form of the deflection depends upon the position of the electrodes in relation to the point of stimulation and the resulting doublets. Therefore, the amount of variation obtained by shifting the electrodes presumably depends upon the relation of the electrodes to the point at which rhythmicity is being built up and discharged. Finally, in record 28 (Fig. 8) where the electrodes were widely separated but not placed at the extreme ends of the muscle strip  the curve is triphasic and thus almost identical with the predicted curve.

Clearly, in these experiments the variation of the form of the electrical complexes in relation to the position of the electrodes is not as regular nor as definite as Craib's experiments might lead one to expect. However, these records were taken from spontaneously beating muscle strips and not from those being driven from a definite point. Therefore, in these experiments one cannot predict the focus at which the contraction was being initiated, nor is that point necessarily the same for every beat. Indeed our tracings show that the impulse may occasionally arise from an "ectopic" focus (see Fig. 3). For these reasons, then, in spite of some discrepancies, we believe it is significant

that certain of the monophasic and diphasic curves obtained in these experiments are quite definitely in accord with those predicted by Craib.

CONCLUSIONS

1. The rhythm observed in isolated strips of cardiac muscle appears to be a true rhythmicity and not due to a re-entrant beat.
2. These curves suggest that their form, i.e., whether it is monophasic or diphasic, depends upon the relationship of the electrodes to the initial point of excitation.
3. Some of the tracings indicate that the origin of excitation of the strip is not always at the same point, but may vary from beat to beat.
4. In one strip alternation in the recovery phase was clearly shown.

In conclusion I wish to acknowledge my indebtedness to Dr. E. P. Carter for his generous help, and valuable suggestions and criticisms. Without them this investigation could not have been carried on.

REFERENCES

(1) Taussig, H. B. and Meserve, F.: *Amer. Jour. of Physiology*, 1925, vol. 72, no. 1, 89–98.
(2) Craib, W. H.: *Heart*, 1927, xiv, no. 1, 71–109.

THE RANGE OF HYDROGEN-ION CONCENTRATION OF CERTAIN BUFFER SOLUTIONS IN WHICH THE CHICKEN-TUMOR VIRUS RETAINS ITS ACTIVITY[1]

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In view of the extensive amount of experimental work that is being undertaken in the study of the behavior of the chicken-tumor virus and the discussion as to whether the malignant principle is a living organism or merely a chemical substance, it is important that we have a more complete knowledge of the range of hydrogen-ion concentration in which the virus can be extracted and in which it will remain active.

As early as 1912, Rous and Murphy found that the causative agent of the chicken tumor remained active when the tissue was either dried and powdered or kept in glycerine, and also that the active principle could be extracted from the tumor tissue by means of salt solution but not by alcohol or chloroform. They likewise found that the causative agent of the tumor was destroyed by a number of different substances, but apparently they did not investigate the effect of acids and alkalies upon the virulence of the virus.

Lewis and Andervont (1928) demonstrated that the virus will remain active in acid (pH 4) and in alkaline (pH 10) solutions of higher and lower hydrogen-ion concentrations that have proved favorable for the survival and growth of cells in tissue cultures. As the addition of acid and of alkali is not a satisfactory method of changing the hydrogen-ion concentration, it was decided to investigate the effect on the chicken-tumor virus of standardized buffer solutions covering a wide range of hydrogen-ion concentration.

The buffer solutions were made up for each set of experiments and none was used a second time. They were as follows: Citrate buffer,

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pH 2, pH 3, pH 4, pH 5, and pH 6 (Sörensen); phosphate buffer pH 6 and pH 7 (Sörensen); mixtures of 0.1 N sodium acetate and varied amounts of acetic acid pH 3.62, pH 4.14 and pH 4.62 (Michaelis); mixtures of hydrochloric acid and potassium acid phthalate pH 3, pH 3.4 and pH 3.6 (Clark); sodium carbonate and hydrochloric acid pH 9.5, pH 10, pH 10.5, pH 11 and pH 11.5; mixtures of secondary sodium phosphate and sodium hydroxide pH 11.2 and pH 11.8, (Michaelis); and sodium hydroxide pH 12, pH 12.5 and pH 13.[2]

The different kinds of buffer solutions were not used the same number of times; 7 experiments were made with the citrate buffer pH 2; 15 with pH 3, 6 with pH 4, 6 with pH 5, and 6 with pH 6. The phosphate buffer pH 6 was used in 6 experiments and pH 7 in 6 experiments. The acetate buffer solutions of pH 3.62, 4.14, and 4.62 and the Clark solutions pH 3, 3.4, 3.6 and 4 were each used for only one inoculation. Sodium carbonate and hydrochloric acid buffers, pH 9.5, 10, 10.5, 11, 11.5 were each used in three inoculations. Buffers pH 11.2 and pH 11.8 were each used in two experiments and sodium hydroxide solutions pH 12, pH 12.5 and pH 13 were each used in four instances.

The tumor used in these experiments was started in 1926 by injecting into a chicken a tumor powder supplied by Dr. James B. Murphy of the Rockefeller Institute for Medical Research. The tumor thus obtained has been transferred from one chicken to another, either by means of transplants or by inoculations of a tumor extract. It has retained its original malignancy during this time, as is shown by the fact that in every instance the injection of 2 cc. of a 1 to 10 extract of a tumor into another chicken has been followed within eight to ten days by the appearance of a malignant growth, which has resulted in the death of the chicken within three or four weeks.

[2] The Clark buffers (pH 3, 3.4, 3.6 and 4), the mixtures of sodium carbonate and hydrochloric acid (pH 9.5, 10, 10.5, 11 and 11.5) and the sodium hydroxide solutions (pH 12 and 12.5) were made up and checked with the hydrogen electrode by the La Motte Chemical Products Company on the same day as they were used in our experiments. All other buffer solutions were prepared and tested by Dr. Michaelis a few hours before the experiment.

EXTRACTION OF THE VIRUS IN BUFFER SOLUTIONS OF DIFFERENT HYDROGEN-ION CONCENTRATIONS

In the first two sets of experiments undertaken in this investigation an effort was made to study the effect of the different buffer solutions on the tumor virus by extracting the tumor in each of the buffer solutions. This plan was soon abandoned, however, owing to the coagulation of the tissue in certain of the buffer solutions. This rendered it impossible to determine, when the virus failed to bring about the growth of a tumor, whether the virus had become inactivated or whether its extraction had been prevented through failure of the solution to penetrate into the tissue. The method was as follows. The firm white part of a tumor was excised from the breast of the chicken, placed in a sterile mortar together with a little sterile glass, and ground to a smooth paste. One gram of the tissue paste was added to 10 cc. of the buffer solution to be tested. The two were mixed and centrifuged, after which 3 cc. of the supernatant fluid, together with a trace of sterile kaolin[3] were injected into the breast of another chicken. The citrate buffer solutions of pH 2, 3, 4, 5 and 6 and the phosphate buffer solutions of pH 6 and 7 were used to extract the tumor tissue. Citrate solutions pH 2, 3 and 4 coagulated the tissue paste, so that before centrifuging it was necessary to grind each of these clots in a separate sterile mortar in order to insure penetration of the fluid into all parts of the tissue. The buffer solutions were made up in the usual way, disregarding the osmotic pressure of the solution, and no attempt was made to alter the osmotic pressure of the buffer solution to conform with that of the tissue. The difference in salt content of the buffer solutions did not injure the virus; it was found to be extracted in citrate solutions having a hydrogen-ion concentration of pH 4, 5, and 6 and in phosphate solutions of pH 6 and 7, since in every instance the chicken receiving the supernatant fluid from the tumor in one of these solutions developed a tumor at the site of the inoculation. Extracts

[3] The injection of a solution containing tumor virus into a chicken is not always followed by the growth of a tumor unless it contains some irritating substance. Rous (1911) showed that the addition of a little sterile Kieselguhr to the filtrate of the chicken tumor assured the growth of a tumor and since that time most investigators have added either Kieselguhr or kaolin to the tumor extract.

of tumor in citrate buffer solutions having a higher hydrogen-ion concentration than these failed to bring about the growth of a tumor when injected into a chicken. Since it was impossible in these cases to be certain that the virus had been inactivated by the hydrogen-ion concentration of the solution or that it had not been extracted because of failure of the solution to penetrate the coagulated tissue, it was decided to prepare the tumor virus in the usual way and then to subject it to the different buffer solutions.

EFFECT OF SOLUTIONS OF DIFFERENT HYDROGEN-ION CONCENTRATIONS ON THE ACTIVITY OF THE VIRUS

In this laboratory it has been the custom to use the chicken-tumor virus in the form of a 1 to 10 extract of the tumor in normal salt solution. As the same approximate strength of virus was to be used in these investigations,[4] it was decided to prepare an extract of twice this strength and then to dilute it one-half with the given buffer solution since such a mixture of virus and buffer solution would not change greatly the hydrogen-ion concentration of the buffer solution. The tumor extract was prepared as follows. To 10 grams of tumor tissue paste were added 50 cc. of sterile salt solution (0.9 per cent NaCl). After this was well mixed, it was poured into sterile tubes and centrifuged for five minutes. The supernatant fluid was then removed and used as the tumor extract. No further effort was made to render the extract entirely free from cellular debris, as it was deemed expedient to use as active a tumor agent as possible. Such a tumor extract has been found to remain active at room temperature for 24 hours and in some instances for 36 hours. Nevertheless, since it has been claimed by some investigators that the chicken-tumor virus becomes attenuated when it remains in salt solution at room temperature for a few hours, the interval between the preparation of the tumor extract and its injection was limited to not more than one hour in order to avoid any possible confusion in our results from such attenuation. In addition to this, the activity of the particular tumor extract used in a given set of experiments was always tested by mixing 5 cc. of it with 5 cc. of

[4] In previous investigations (Lewis and Andervont, 1927 and 1928), tumor extracts prepared in this manner never failed to bring about the growth of a tumor in the more than a hundred chickens into which they were injected.

physiological salt solution and permitting it to remain until the experiment was completed, after which this diluted tumor extract was injected into a chicken.

In each set of experiments the buffer solution was prepared and sterilized a few hours before the tumor extract was made. As soon as the tumor extract was centrifuged, 5 cc. of it were added to 5 cc. of the buffer to be tested. The two were mixed by shaking gently; then 3 cc. of the mixture, to which a little sterile kaolin had been added, were injected into the breast of a chicken. In order to determine whether the change in hydrogen-ion concentration of the solution affected the virus immediately or gradually, some of the mixtures of buffer solution and tumor extract were allowed to stand for definite periods of time—five, ten, fifteen, twenty, thirty and forty-five minutes—before being injected. When it was found from these experiments that the buffer solution had the same effect upon the tumor virus whether the mixture stood for forty-five minutes or only for five minutes, no effort was made to limit to an exact interval the time between the mixing of the two solutions and their injection, except that it was kept as short as possible. The interval was usually about fifteen minutes, and never longer than thirty minutes, depending upon the number of buffer solutions to be tested in a given experiment. When an alkaline buffer solution was to be tested, the mixture of buffer solution and tumor extract was injected as quickly as possible. It is not advisable to let alkaline buffer solutions stand in contact with the air, as the hydrogen-ion concentration is apt to change by absorption of carbon dioxide from the air. In some instances the tumor extract and the alkaline buffer solution were mixed in a hypodermic syringe and injected at once, but no difference was noted between the results thus obtained and those in which the mixture was allowed to stand as long as ten minutes.

A different tumor was used for the preparation of the extract in each set of experiments. In all, 14 tumors were used. The extracts did not differ in activity to any noticeable extent, as the inoculation of the control diluted tumor extract at the end of each set of experiments was followed by the growth of a malignant tumor within eight to ten days. Many of these control tumors were used for the preparation of the tumor extract in later experiments, but the few that were not

so employed brought about the death of the chicken within a month. There was, however, one exception to this, that of chicken no. 27, which will be referred to later on.

A mixture of equal parts of tumor extract and a citrate buffer solution of pH 2 was injected into the breast of 5 chickens, none of which developed a tumor. A mixture of tumor extract and a citrate buffer of pH 3 was injected into 13 chickens, and in none of these did a growth of a tumor follow. On the other hand, the injection of mixtures of tumor extract and citrate buffers of pH 4, pH 5 or pH 6, each of which was tried in 4 experiments, was followed by a tumor in all of the 12 inoculations. A mixture of tumor extract and acetate buffer pH 3.63 inactivated the tumor virus, while mixtures of tumor virus and acetate buffer pH 4.14 and pH 4.63 failed to inactivate the virus and the injections were followed by the growth of a tumor. Mixtures of the tumor extract and Clark buffers of pH 3, 3.4, and 3.6 all failed to bring about the growth of a tumor. The addition of a phosphate buffer of pH 6 or pH 7 to a tumor extract never inhibited the activity of the virus, and every one of the 8 chickens receiving the tumor extracts in these solutions developed a tumor. The virus was not inactivated by buffer solutions of pH 9.5, 10, 10.5, 11, 11.2 or 11.5 and each of the 18 inoculations of these mixtures resulted in a large and malignant tumor. The mixture of secondary sodium phosphate and sodium hydroxide of pH 11.8, however, did inactivate the tumor virus in one instance. The addition of sodium hydroxide pH 12 to the tumor virus inactivated the virus in one instance, but in three other experiments the inoculation of a mixture of tumor extract and sodium hydroxide pH 12 was followed by the growth of a tumor. In the four instances in which the mixture of sodium hydroxide solution of pH 12.5 and a tumor extract was injected, the chickens failed to develop a tumor. In four experiments an equal part of a one-tenth normal solution of sodium hydroxide (about pH 13) was added to the extract and none of the chickens receiving this mixture developed a tumor.

In a few experiments the hydrogen-ion concentration of the citrate buffer solution and tumor extract mixture, as well as that of the mixtures of phosphate buffer solution and tumor extract, was tested after the experiments were completed and the corrected hydrogen-ion concentrations recorded. These did not differ greatly from those of

the original buffer solution. The mixture of tumor virus and citrate buffer pH 2 in one experiment had become pH 2.50, in another, pH 2.51 and in a third, pH 2.43; the mixture with citrate buffer pH 3 had become in one experiment pH 3.13, in another pH 3.13, and in a third pH 3.34; the mixture with citrate buffer pH 4 in one experiment had become pH 3.90, in another pH 3.92 and in a third pH 4.00; the

TABLE I

NUMBER OF INOCULATIONS	KIND OF BUFFER	pH	NUMBER OF CHICKENS THAT DEVELOPED A TUMOR
7	Sörensen citrate	2	0
15	Sörensen citrate	3	0
1	Clark	3	0
1	Clark	3.4	0
1	Clark	3.6	0
1	Acetic	3.62	0
6	Citrate	4	6
1	Acetic	4.14	1
1	Acetic	4.62	1
6	Sörensen citrate	5	6
6	Sörensen citrate	6	6
6	Sörensen phosphate	6	6
6	Sörensen phosphate	7	6
3	Na_2CO_3 and HCl	9.5	3
3	Na_2CO_3 and HCl	10	3
3	Na_2CO_3 and HCl	10.5	3
3	Na_2CO_3 and HCl	11	3
3	Na_2CO_3 and HCl	11.5	3
2	Secondary Na phosphate and NaOH	11.2	2
2	Secondary Na phosphate and NaOH	11.8	1
4	NaOH	12	3
4	NaOH	12.5	0
4	NaOH 0.1N	13	0

mixture with citrate buffer pH 5 in one experiment had become pH 4.75, in another pH 4.75, and in a third pH 4.81; and the mixture with citrate buffer pH 6 in one experiment had become pH 5.58, in another pH 5.61, and in a third pH 5.56. The mixture of tumor virus and phosphate buffer pH 6 in one experiment had become pH 6, in another pH 6.03, and in a third pH 6; the mixture with phosphate buffer pH 7 in one experiment had become pH 6.98, in another pH 7.00, and in a

third pH 6.98. The control mixture of tumor extract and physiological salt solution was in one experiment pH 6.92, in another pH 6.60, and in a third pH 6.80.

The experiments are summarized in Table I.

Naturally we were interested in determining whether the injection of a tumor virus inactivated by means of one of these buffer solutions conferred an immunity. None of the chickens that had failed to develop a tumor following the injection of a mixture of tumor virus and buffer solution, however, were found to be immune to a later transplant of a tumor. On the contrary, when a small fragment of tumor was grafted into the breast muscle, it promptly developed into a growth, which ultimately resulted in the death of the host.

An effort was also made to ascertain whether a tumor once started could be caused to regress by injections of the buffer solutions. Four chickens were given small transplants into the breast muscle; when these had reached a palpable size, the chicken was given a daily injection of 5 cc. of a citrate buffer of pH 2. This retarded the growth of the tumor but did not bring about its regression, and while these chickens lived much longer than the control, they ultimately died. Death was due in part to the tumor and in part to the severe anemia which had developed in these chickens.

In these experiments 2 chickens (nos. 27 and 30) developed small tumors which later regressed, leaving the chickens immune to further inoculations. Chicken 27 received on one side a mixture of tumor extract and salt solution. Chicken 30 received on one side a mixture of tumor extract and citrate buffer pH 4 and on the other side a mixture of tumor extract and phosphate buffer pH 6. Chicken 27 developed a tumor on the left, which reached a palpable size at the end of ten days and remained so for twenty days, after which it rapidly regressed and the chicken appeared normal at the end of a month. Chicken 30 developed a tumor about the size of a pea on the right side and a larger one on the left. The tumor on the right regressed after twenty days; the one on the left remained for thirty days, after which it rapidly decreased in size and had entirely disappeared at the end of forty days. Each of these two chickens was given a transplant fifty days after the first inoculation, but both failed to develop tumors. At the time they were grafted, transplants of the same tumor were given to

6 other chickens, all of which developed tumors resulting in death. Chicken 27 and chicken 30 were each given a second transplant ninety days after the first inoculation and another chicken was given a transplant at the same time. The control chicken developed a tumor, but neither no. 27 nor no. 30 did so. They were therefore judged to be immune.

SURVIVAL AND GROWTH OF TISSUE CELLS IN THESE BUFFERS

In recent years a number of investigations have been made on the relation of hydrogen-ion concentration to the growth of tissue cells. Rous (1913) colored plasma blue with litmus and explanted into it pieces of embryonic tissue and of chicken tumor. The pieces that did not grow remained blue, while those that did grow became pink, showing that they were acid. Levaditi and Gabrek (1914) stated that tissue stained with neutral red and explanted into plasma became yellow when sterile and colorless after vigorous growth. Lewis and Felton (1921) used nutrient salt solution in place of plasma. They found that embryonic chick tissue exhibited growth when explanted into nutrient solutions having hydrogen-ion concentrations between pH 5.5 and pH 9, but the medium most favorable for the growth of these cells was one of about pH 6.8 or 7. Tissues explanted into media of pH 4 to pH 5.2 did not grow and those in solutions more alkaline than pH 9 seldom grew. Fischer (1921) grew tissue for as many as six generations in plasma pH 5.5 and as many as ten generations in plasma pH 8.5, while the optimal hydrogen-ion concentration was 7.4 to 7.8. Mendéléef (1923) claims that the most favorable medium for growth of embryonic guinea-pig tissue was obtained by bringing about an acidity of pH 5.8 to 6 in plasma. This was most successfully done by freezing the plasma or by injecting milk into the animal from which the plasma was to be obtained. Radzimowska (1923) placed pieces of rabbit spleen for half an hour in Ringer's solution containing various acids and then, after washing them, explanted them into normal plasma. He found that organic acid was more toxic than inorganic acid and that some migration of cells occurred even after exposure of pieces of spleen for half an hour to sulphuric acid pH 3.1, hydrochloric acid pH 3.15, phosphoric acid pH 3.1, citric acid pH 4.05, lactic acid pH 4.21, or acetic acid pH 5.29.

According to our experience it is likely that the apparent resistance of the spleen tissue for relatively strong acid solutions was due to the fact that the acid produced a coagulation on the surface layer of the tissue piece and so did not diffuse into the center of the pieces out of which later uninjured cells migrated. Harde and Danysz (1925) found that the pH of growing tumor cells explanted in litmus plasma lay about pH 6.

Since none of the investigations were quite comparable to the experiments carried out with the tumor virus, it was decided to prepare a series of tissue cultures, exposing the tissue to the same buffer solutions and for the same length of time as was done with the tumor virus. Four sets of cultures were made. In the first set, pieces of skin from an 8-day chick embryo were excised and one placed in 5 cc. of each of the citrate buffer solutions of pH 2, pH 3, pH 4, pH 5, and pH 9, phosphate buffer solutions of pH 6 and pH 7, and 0.1 N sodium hydroxide solution. Each piece of skin was then cut into ten small pieces and allowed to remain in the solution for fifteen minutes, after which these were explanted into nutrient salt solution in the usual manner. None of these cultures exhibited any growth, although the control pieces, which had been cut up and left in the nutrient solution pH 7, all grew vigorously. The second set of cultures was a duplicate of this except that a different lot of buffer solutions of the same hydrogen-ion concentrations were used. The results were the same; hence it was evident that the tissue must have been injured by some other property of the buffer solution (specific chemical action of the chemicals or inadequate osmotic pressure) than the hydrogen-ion concentration, as some of the solutions (citrate pH 6 and phosphate pH 7) lay within the range of hydrogen-ion concentration favorable to growth.

In the third series of cultures the pieces of tissue were cut up in equal parts of physiological salt solution and buffer solution, shaking gently to insure mixing of the two fluids. After the tissue had stood in the mixture of buffer and salt solution for fifteen minutes, the pieces were explanted into the nutrient salt solution. The buffer solutions used in these cultures were phosphate buffers pH 8, pH 7.4, pH 7, pH 6 and pH 5.5. Ten cultures were made from each of the solutions and all sixty exhibited a large and vigorous growth.

In the fourth set of cultures pieces of skin were removed from an

8-day chick embryo and one piece was placed in 5 cc. of each of the following buffer solutions: citrate buffer pH 3, pH 4, pH 4.96, pH 6 and acetate pH 4.62. In addition to these solutions, pieces were placed in mixtures composed of equal parts of nutrient salt solution and buffer solution and one piece in a control solution consisting of equal parts of nutrient salt solution and normal salt solution. The addition of the nutrient salt solution changed the hydrogen-ion concentration of the buffer solutions from 3 to 3.55; 4 to 4.11; 4.96 to 4.93; 6 to 5.86; 4.62 to 4.64; and the control solution was pH 6.83. The pieces of tissue were cut up in the solution and after fifteen minutes were washed in nutrient salt solution and explanted in the usual way. None of the pieces cut up in the buffer solutions alone exhibited any growth, even at the end of four days. The pieces that had been cut up in a mixture of salt solution and buffer solution of pH 4 or higher likewise failed to grow. The pieces cut up in a mixture of salt solution and citrate buffer pH 4.96 and of salt solution and citrate buffer pH 6 grew vigorously and at the end of four days the growth was as large and as healthy as that of the controls. The pieces cut up in a mixture of salt solution and acetic buffer pH 4.62 failed to grow.

DISCUSSION

The authors are conscious of the difficulties involved in attempting to solve the problem concerning the influence of hydrogen-ion concentration on living systems. In order to study the influence of a varied hydrogen-ion concentration, various solutions should be applied that are absolutely alike except for the H^+ concentration. This is impossible, of course, since any two solutions that differ with respect to H^+ ions must also differ with respect to some anions with which the H^+ ions are in equilibrium. This primary condition not being obtainable, it should at least be shown in any particular case that, within reasonable limits, the nature and concentrations of all ions of the buffer except the H^+ ions are of no significance. This was done in the studies on the action of pH upon certain enzymes (*e.g.*, for invertase by Sörensen (1909) and by L. Michaelis (1911)) and it was this fact alone that enabled us to speak of a definite effect of the hydrogen ions. In the present experiments with tumor extract and cells, the unequivocal influence of the H^+ ions cannot be easily proved. In the case

of chick embryo cells, it could even be shown that the H^+ ions are certainly not the only determining factor, since buffer solutions after dilution with saline acted very differently, without being appreciably changed in pH. Despite these difficulties, however, the fact remains that the tumor extract showed a behavior towards certain buffer solutions different from that of embryonic cells and that the effect of buffer solutions on tumor extracts did not appreciably vary with the electrolyte content of the applied buffers as far as could be observed. Keeping in mind this restriction, it should not lead to a misinterpretation if we state, as a result of these investigations—

(1) That the virus has a resistance for H^+ ions between 4 and 12.

(2) That the nature of buffer by which this pH is produced appears to be of no great importance.

(3) That embryonic cells have, under favorable conditions of the buffer, a resistance for a pH between 5 and 9.

(4) That for embryonic cells the nature of the buffer is very important, too, in that any unfavorable condition of the buffer (disregarding the H^+ ion) may just as well be harmful to the cell as an inadequate range of hydrogen-ion concentration.

In this respect the virus is somewhat similar to bacteria, most of which tolerate also somewhat the same range of hydrogen-ion concentration, according to species. No protozoon showing such resistance of hydrogen-ion concentration has so far been described, even those having the widest range (Colpidium campylum, pH 4.5 to 9, according to Bresslau (1926)).

As to whether the virus might be a compound, such as an enzyme, its resistance to change in pH is compatible with this view, but the fact that substances like chloroform, alcohol, toluol (Rous and Murphy), acetone (Nakahara), thymol (unpublished), H_2O_2 (unpublished), etc., inactivate the virus irreversibly in a short time finds no analogy in the most careful studies of enzymes so far made. Pending more extended investigations along these lines we may state that the chicken-tumor virus, in respect to its resistance to chemicals, shows a behavior very different from that of an animal cell or a protozoon; it is comparable rather to bacteria, provided of course, it is a living organism.

CONCLUSIONS

The chicken-tumor virus remained active in buffer solutions having a hydrogen-ion concentration of 4 to 12, but became inactive in buffer solutions of pH 3 and in sodium hydroxide pH 12.5.

REFERENCES

BRESSLAU, E. 1926 Die Bedeutung der Wasserstoffionenkonzentration für die Hydrobiologie. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie*, Bd. III, S. 56–108.

FISCHER, A. 1921 Growth of fibroblasts and hydrogen-ion concentration of the medium. *Jour. Exper. Med.*, vol. 34, p. 447.

HARDE, E., AND M. DANYSZ. 1925 Sur la réaction acide des tumeurs. *C. r. Soc. biol.*, t. 93, p. 1489.

LEVADITI, C., AND F. GABREK 1914 Sur la vie et la multiplication *in vitro* des cellules préalablement colorées. *C. r. Soc. biol.*, t. 77, p. 417.

LEWIS, M. R., AND H. B. ANDERVONT 1928 The inactivation of the chicken-tumor virus by means of calcium compounds. *Bull. Johns Hopkins Hospital*, vol. 42, p. 191.

LEWIS, M. R., AND L. D. FELTON 1912 The hydrogen-ion concentration of cultures of connective tissue from chick embryos. *Science*, vol. 54, p. 636.

MENDÉLÉEF, P. 1923 Les cultures de tissue embryonnaire de cobaye dans les milieux de pH déterminés. *C. r. Soc. biol.*, t. 88, p. 291.

MICHAELIS, L., AND H. DAVIDSOHN 1911 Die Wirkung der H-ionen auf das Invertin. *Biochem. Ztschr.*, Bd. 35, S. 386.

NAKAHARA, WARO AND MIZUHO SUMI 1927 The alleged enzyme-like nature of the so-called causative agent of chicken sarcoma. *Proc. of Imp. Acad.* Bd. 3, S. 9, 376.

RADZIMOWSKA, V. 1923 Die Wirkung verschiedener Säuren auf die Gewebezellen warmblütiger Organismen. *Biochem. Ztschr.*, Bd. 142, S. 36.

ROUS, P. 1913 The growth of tissue in acid media. *Jour. Exper. Med.*, vol. 18, p. 183.

ROUS, P., AND J. B. MURPHY 1912 The nature of the filterable agent causing a sarcoma of the fowl. *Jour. Amer. Med. Assoc.*, vol. 58, p. 1938.

SÖRENSEN, S. P. L. 1909 Enzymstudien II. *Biochem. Ztschr.*, Bd. 21, S. 131.

THE MAIN CHARACTERISTICS OF CARCINOMA CELLS IN VITRO[1,2]

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I. One year ago we succeeded in developing a method by which mammalian carcinoma cells could be cultivated indefinitely *in vitro*. The strain of mouse carcinoma cells has proved to be ideal for physiological investigation and has already, I believe, contributed a good deal to our knowledge of the biology of tumor cells. This strain is also ideal because of the simplicity with which it can be cultivated. Carcinoma cells are easier to cultivate than normal tissues on account of the relative ease with which they become adapted to their environment. I shall endeavor at this time to outline a few of the experimental facts which have resulted from our studies.

The carcinoma cells were derived from the well-known Ehrlich mouse carcinoma (Frankfurter strain), which is an adenocarcinoma. The cultivation technique is mainly the same as that which I devised five years ago for the indefinite cultivation of the Rous-sarcoma. These sarcoma cells—as you know from the work of several investigators—have the unfortunate property of producing an extensive degree of action in the plasma medium. For this reason, it was necessary to add small pieces of dead or living normal tissues side by side with the sarcomatous tissue in order that transference of the cultures from one medium to another would be possible. For the sarcoma cells it was a *conditio sine qua non* that such a piece of tissue should be added. For the carcinoma cells, however, it was found that an addition of tissue was not absolutely necessary since the cells do not liquefy the medium, though it exercises a certain influence, as we shall see later.

Briefly, the medium employed in the cultivation of the carcinoma cells consists of one volume of chick embryonic tissue juice and one

[1] Lecture delivered at the Johns Hopkins Medical School, February 27, 1928.

[2] Received for publication April 5, 1928.

volume of a plasma mixture composed of 25 per cent of chicken plasma and 75 per cent of rat plasma. Pieces of mouse tissue, either fresh or stored for a long time in the ice-box, are brought in contact with the carcinomatous tissue. After from 2 to 4 passages the normal tissue is found to be infiltrated throughout with carcinoma cells. If the normal tissue is added in a living condition, an outgrowth of normal cells can naturally be seen, but after some passages this disappears entirely, leaving only carcinoma cells. The strain of carcinoma cells is now one year old and has maintained its full malignancy over the whole time.

II. But heterologous tissues can be used with the carcinoma cells as well as homologous tissues. In no case has it been found that the normal cells are able to overgrow the carcinoma cells. This, in itself, is a characteristic of great importance. If a piece of normal fibroblast culture is added to a pure culture of epithelial cells, the fibroblasts within a short time will overgrow the epithelium, which finally dies. If a few carcinoma cells are added to a large culture of fibroblasts, the carcinoma cells will progressively cause the death of the fibroblasts. *It makes absolutely no difference what kind of or how many normal cells (connective tissue, epithelium, muscle or cartilage) are added to carcinoma cells, the carcinoma cells will eventually overgrow any tissue in vitro.* This is only a demonstration of our definition of cancer,—a tissue which is not subject to the laws of regulation and coördinate growth in the organism, but which continues to proliferate regardless of the limits of growth of the various tissues with which it comes in contact. This phenomenon can be explained on the assumption of the theory of the desmones, *i.e.*, the growth principles which are transported directly from one cell to another and which are responsible for the correlation of the growth of the various tissues in the body. Epithelial cells cannot utilize the desmones of the fibroblasts, and *vice versa*. They are highly specific in their action as to cell types and species of animal. Tumor cells, however, are able to utilize any desmones in the body. This fact explains the heterotopic growth of the cancer cells. The exact nature of these growth principles, the desmones, is at the present time unknown, but their presence can be demonstrated through their action.

III. The growth of the carcinoma cells can be accurately measured in the usual way, since they do not liquefy the culture medium. The two halves of a carcinoma culture grow under identical conditions so regularly that their growth difference is seldom greater than 5 per cent.

The rate of growth of carcinoma cells is slower than that of normal homologous and heterologous cells under conditions which are optimal for the normal cells. It has been found by Carrel and Ebeling that a fibroblast colony doubles its mass within 48 hours, and a colony of epithelial cells in 72 hours, whereas a carcinoma colony requires a longer time to double itself.

The explanation for the continued proliferation of cancer cells in the body among dormant cells cannot, therefore, be found in any power which they may possess to proliferate faster than normal cells *in vitro*—such a power we find they do not possess. In the body the normal cells, which have been quiescent for years, are always able to multiply, and even much faster than many tumor cells, but under certain conditions, *e.g.*, wound healing.

Cultures of normal tissue cells require the addition of embryonic tissue juice or proteoses (as discovered by Carrel and by Carrel and Baker) to the plasma medium before an increase in their mass becomes possible. In serum alone the cells die after a time, and growth, while it lasts, is only at the expense of nutrient substances in the explanted tissue itself.

In the meantime, we found that the carcinomatous epithelium is able to grow in pure rat serum as the only nourishment, and in embryonic tissue juice inactivated by subjection to a temperature of 56°C. for one hour, a treatment which renders it useless for fibroblasts and epithelium. This indicates that the carcinoma cells are capable of utilizing the substances present in embryonic tissue juice in a form different from that required by the normal cells.

IV. If we measure the rate of growth of carcinoma cells as a function of the concentration of pure embryonic tissue juice and proteoses in the pericellular medium, we find that they behave in that respect almost as do leucocytes, as was formerly demonstrated by Carrel and Ebeling. In 100-per-cent embryonic tissue juice, the cells become

filled with vacuoles and granules, the medium becomes liquefied and death follows. In 40-per-cent they grow a little better and for a somewhat longer time, but the cells become vacuolated as before. The proteoses, we found, have the same effect on the carcinoma cells as the embryonic tissue juice.

V. At the present time it has become possible to measure the residual growth energy of the carcinoma cells. Some precautions have to be taken on account of the fact that the carcinoma cells are able to live on the serum constituents of the plasma. It is therefore necessary to wash out almost all of the serum from the clot before beginning the measuring process.

We have found that the residual energy of the carcinoma cells is lower than that of normal tissue cells, and we also found that this residual energy varies with different cultures. But what is especially interesting is the fact that it also varies inversely with the length of time which has elapsed since the addition of normal tissue to the culture. When a piece of normal tissue is added, the residual energy is more pronounced than it is after a long period has elapsed since the last addition. However, this variation in the rate of growth is not very great. When, on the other hand, the carcinoma cultures are cultivated in pure serum, the differences in the growth rate are every striking. Cultures to which a piece of tissue has been recently added show a very high growth rate, whereas cultures which have not received any normal tissue for a long period show a much lower rate of growth.

These experiments demonstrate the fact that the carcinoma cells receive certain principles from the normal tissue cells with which they come in contact, substances which in themselves provide no nourishment. This we know from the fact that, if the carcinoma cells are kept in a protective salt solution, *e.g.*, Tyrode, they grow only for a very short time and then die, whereas if cultivated in pure serum their growth is regular and abundant. The rate of growth is therefore regulated by a certain accessory factor acquired from the normal cells. It seems reasonable to regard this factor as a sort of activator which probably accelerates the metabolism of the cells and is identical with the desmones.

VI. Several years ago Rous found that the chick sarcoma, when cultivated *in vitro*, produced acid. We now know, from the experiments of Warburg, that this is lactic acid, and that its production is due to the high glycolytic power of all tumor cells. Carcinoma cultures are more sensitive to changes in the hydrogen-ion concentration of the medium than are cultures of normal tissues, and are far less resistant to changes on the acid side. In fact, it is possible, in a mixed culture of normal cells and carcinoma cells, to check the growth of the latter by merely increasing the hydrogen-ion concentration. $CaCl_2$ will bring about the same effect.

VII. Some years ago, we found that tumor cells are more sensitive to increased oxygen tension than are normal cells. Accordingly, it became of interest to study quantitatively the effect of low oxygen tension on the growth of tumor cells. For the preliminary experiments the simple technique, well known in bacteriology, of cultivation in high layers of culture medium was used. It was rather surprising to find that the carcinoma cells were far more sensitive to lack of oxygen than the normal cells. The cancer cells died at a distance from the free surface where normal epithelium and fibroblasts proliferated vigorously. Nor did the addition of large amounts of glucose improve the growth of the cancer cells at low oxygen tension. These experiments indicated that a relatively high oxygen tension is necessary for the decomposition and partial elimination of the lactic acid produced in the cultures. For we know from the experiments of Warburg that under anaërobic conditions the tumor cells become more acid. It is quite probable that this accumulated acid inhibits the growth and causes the death of the cells.

VIII. We may briefly summarize these findings, as follows:

1. Carcinoma cells are able to proliferate indefinitely among the dormant cells in the organism—(*a*) because of certain humoral factors. The cells are able to build up unlimited masses of protoplasm from the serum constituent; and (*b*) because of certain cellular factors the cells are able to utilize the otherwise cell-type-specific growth principles, the desmones, from any cells in the organism. This fact explains the heterotopic growth of cancer cells in general.

2. The rate of growth of tumor cell *in vitro* is markedly lower than that of the normal tissue cells under conditions which are optimal for the latter.

3. Carcinoma cells are able to build up their cytoplasm from embryonic tissue juice which has been inactivated by subjection to a temperature of 56°C. for one hour. This indicates that carcinoma cells are able to utilize certain substances in a form different from that required by normal cells.

4. Normal tissue cells are unable to overgrow carcinoma cells *in vitro*.

5. Carcinoma cells are more sensitive to an increased hydrogen-ion concentration that are normal tissue cells.

6. Carcinoma cells are more sensitive to high and low tensions of oxygen than are normal tissue cells.

7. The growth of carcinoma cells ceases at a low oxygen tension, whereas, under similar conditions, normal tissue cells continue to proliferate.

BOOK REVIEWS

Osler's Modern Medicine, Third Edition, re-edited by Thomas McCrae, M.D., assisted by Elmer H. Funk, M.D. Vol. IV. Diseases of the Respiratory and Circulatory Systems. Pp. 1011. $9.00. (*Lea and Febiger, Philadelphia, 1927.*)

The fourth volume of a very popular System of Medicine comprising presentations of Diseases of the Respiratory and Digestive Systems. It is gratifying to note the maintenance of the high standard set by previous editions. The arrangement of the material is much the same but with evidences of thorough revision. One finds a happy combination of both theoretical and practical information with stress upon the details of treatment in many instances. A very concise chapter on the "Physiology of Respiration" elucidates much of the modern knowledge of this important subject. Diseases of the heart are considered in the usual manner on the basis of anatomical divisions of the heart rather than on the basis of etiology. The second manner of presentation has always appealed to the reviewer as the more logical one. Aneurism, a heretofore notable store-house of facts and diagnostic data, is still largely preserved in its attractive style. Dr. Maude Abbott's meticulous account of congenital heart disease constitutes just two hundred pages of this volume. This System will undoubtedly continue to enjoy its well merited popularity among the other American Systems of Medicine.

E. H. H.

Clinical Case Taking, Supplement to Methods in Medicine. By GEORGE R. HERRMANN, M.D. Pp. 90. $1.50. (*The C. V. Mosby Company, St. Louis, 1927.*)

A short treatise dealing with history taking and physical examination in which the aim of the author is to present a somewhat complete outline of the art. The work gives evidence of careful thought and preparation. Each symptom is recorded, followed by lists of important data to be recorded under the various headings. A complete outline of the neurological examination is included together with a brief mention of adequate laboratory tests to be done in certain groups of cases. A valuable appendix is presented including charts representing; (*a*) anatomic outlines conforming to habitus types, (*b*) regions and surface landmarks of the body and (*c*) skin and segmental nerve distribution on the body surface.

E. H. H.

Animal Biology. By J. B. S. HALDANE and JULIAN HUXLEY. 344 pp. $2.50. (*Oxford University Press, 1927.*)

This is one of the Clarendon Series of popular science readers designed to meet the demand of the "science for all" movement. The well-known authors (like

the grand-uncle of the junior author) combine the ability to write so as to be understood with outstanding achievement in physiological and biological research. In their hands this further attempt to present *multum in parvo* to the general reader and the beginner in biology is expected to meet with success. The expectation has for the most part been realized.

After briefly acquainting the reader with a typical vertebrate, the frog (a plan adopted in numerous "Zoology I" courses), Development and Heredity are skilfully presented. Evolution and the phylogeny of the Animal Kingdom, dealt with in the last four chapters, are further fields in which the junior author is thoroughly at home. The matter in between is physiological and has as its central concept Energy, its sources and exchanges. Regulation by nerve paths and by the blood stream is treated according to modern ideas of the nervous mechanism and the endocrine and other chemical relations in the body. Chapter IX, on "Some Points in the Physiology of Development," affords an only too brief introduction to the results of regeneration and transplantation experiments in lower forms.

Since the work aims to "put over" general biological principles, illustrative cases are reduced to the minimum necessary and are well selected. Among the excellent figures some of us "old timers" are pleased to recognize *clichés* that harken back to the elder Huxley's "Lessons" and to the "Human Body," by Newell Martin, a former Johns Hopkins Professor and at one time a student of Thomas H. Huxley.

C. G. H.

Diagnosis and Treatment of Diseases of the Stomach, with an Introduction to Practical Gastro-Enterology. By MARTIN E. REHFUSS, M.D. With 519 Illustrations; some in colors. 1236 pp. $12.00. (*W. B. Saunders Co., Phila., 1927.*)

The author approaches his subject in an original way, and develops it thoroughly; the knowledge displayed and the advice given in the first two chapters provide especially interesting and instructive reading.

The chapters: "Anatomy and Embryology," and "Physiology of the Stomach," are comprehensive and clearly presented.

The subject of gastric technique—food digestion in the stomach, with a detailed presentation of the various curves in the fractional gastric analysis, and of chemical technique, as developed by the author,—is discussed minutely; possibly the author had devoted too many pages to a theme that might be thoroughly covered more concisely.

The chapter on x-ray examination of the stomach is likewise thorough, but the technical details regarding apparatus, film-technique, arrangement of the fluoroscopic chamber, and so forth, would seem to be superfluous, and their elimination would not detract in any way from the value of the book.

In regard to the etiology, pathology, symptomatology, diagnosis and treatment of various diseases, the author's presentation is excellent; and the chapter on

"Medical Consideration of Post-operative Gastric Conditions" is to be particularly commended.

There are many other sections,—notably those devoted to special subjects such as "The Stomach and Diseases of the Nervous System," which are excellent additions to a book of this type.

On the whole, it may be said that Rehfuss has compiled a splendid array of informative knowledge and has presented it in an intelligent and helpful, as well in a comprehensive manner. The book is unhesitatingly recommended both to internists and students.

E. H. G.

Practical Bacteriology, Blood Work and Parasitology. By E. R. STITT, M.D. 8th Edition, 837 pp. $6.00. (*P. Blakiston's Son & Co., Philadelphia, 1927.*)

This, the latest edition of Admiral Stitt's well-known book, will be welcomed by all those with whom the earlier editions have found favor. The author in his preface states that when he set out to revise the previous edition it was his intention to reduce the size of the latter by 100 pages, but when the task was finished, the new edition was larger than the old by about the same amount. If he is no more fortunate with future revisions, his book will soon comprise 1000 pages.

It is doubtful if there is any other single volume in the English language which is so packed with information useful for the clinical bacteriologist, clinical pathologist and clinical chemist, and if it is necessary or advisable to attempt to cover all three fields in one volume, the reviewer knows of no book that serves the purpose better. Admiral Stitt's volume bears all the ear-marks of a manual written by one who is thoroughly familiar with the subject. The portions dealing with laboratory procedures constitute the best in the book and entitle it to the warmest commendation. The clinical notes might profitably be left out of future editions if the author is anxious to keep his volume from becoming too large.

A. M. C.

Cardiac Arrhythmias. Clinical Features and Mechanism of the Irregular Heart. By IRVING R. ROTH, M.D. 210 pp. $7.50. (*Paul B. Hoeber, New York, 1928.*)

As indicated in the introduction by Dr. Emanual Libman, this book is essentially a presentation of many graphs, diagrams and actual electrocardiographic and pulse tracings used by the author in teaching the mechanism of the cardiac arrhythmias. In no sense is it an exhaustive treatise on the subject, the text being sufficient only to explain the graphs, diagrams and tracings. The anatomy and physiology of the heart, with special reference to the specialized conducting tissue, are briefly discussed, and an attempt is made to emphasize those clinical features of the arrhythmias which render possible their recognition without instrumental aid. Etiology, prognosis and therapy are but briefly touched upon, but the author has succeeded admirably in his main object—the elucidation of

the mechanisms of the arrhythmias so that they may be readily understood by student and physician.

The diagrams are clear and are especially valuable in that they portray schematically the sequence of conduction, electrocardiographic tracing, heart sounds and jugular and carotid pulse waves incident to each cardiac cycle in each type of arrhythmia. The free interchange of the terms "extrasystole" and "premature beat" tends to be confusing and one might suggest the use of the term "ectopic arrhythmia" in place of "extrasystolic arrhythmia." Exception might also be taken to the statement that the venous pulse is of no diagnostic value in the paroxysmal tachycardias. On the whole, the author is conservative in his statements and the book can be recommended highly as an easily-readable, clear treatise on the subject.

B. R. H.

Special Cytology. Edited by E. V. Cowdry, The Rockefeller Institute for Medical Research, two volumes; vol. 1, pp. 780, vol. 2, pp. 1325. Cloth. $20.00. (*P. B. Hoeber, 1928.*)

These two volumes illustrate the growing tendency towards cooperation of a group of workers in the production of scientific handbooks. Each subject is allotted to an investigator, who through his own personal researches possesses intimate knowledge of the particular task assigned to him. Great latitude is shown in the choice of contributors. Recruited chiefly from the medical sciences, representing a variety of interests and specializations, one discovers among them pathologists, surgeons, zoologists, internists and anatomists. Such a plan brings to light secular emphasis in some instances; but if this be a criticism, it is overshadowed by the manner of subject presentation and the variety of literary styles, which dispel a certain monotony attached to volumes of similar magnitude written by a single author. Moreover, because of this plan of "division of labor," there is a reduction in second-hand information and more present-day knowledge is included.

Designed for the purpose of presenting the latest concepts of cytology to the student of biology and medicine, comparatively no limitation of approach is imposed; thus, perspective and increased interest are obtained by including some comparative anatomy, embryology and considerable pathology. To the reviewer, the outstanding accomplishment is the continued attempt to correlate cell structure with function: a critical survey of accumulated evidence attributing a physiological rôle to the various normal intra-cellular constituents particularly, and less so to their alterations induced by various experimental and diseased conditions.

In a stimulating introduction, Alexis Carrel reviews the advances of cellular investigation, emphasizes the benefits attained by studying living tissues, makes a plea for the further development of improved methods of technique, and leaves us with the impression that, though great advances have been achieved in the past few decades, much more remains before us.

Contained in the first volume are the sections on the skin, the respiratory and

alimentary systems, the blood, the endocrine organs, and the kidneys. One or more sections have been devoted to one system, depending on the number of functionally distinct tissues; for example, the alimentary system is subdivided into sections on the salivary glands, the gastric glands, the liver, the pancreas and the intestinal epithelium. Relatively much space is given to discussions of the blood cells, wherein the description of each cell type is entrusted to a different author. The mono- and poly-phyletic views concerning the origin of blood cells are propounded in almost adjacent pages. E. B. Krumbhaar, in a lucid description of the erythrocyte, adheres to the latter school, lately strengthened by Sabin and her co-workers, while A. A. Maximow, in a detailed, rather extensive review, discusses the macrophages, the lymphocytes and plasma cells from the unitarian standpoint. One still meets a troublesome complexity of nomenclature, particularly in reference to primitive cell types, which Krumbhaar clarifies greatly by a chart. Hal Downey advances several challenging conclusions on the potentialities of the myeloblast, assuming that it may differentiate normally into granulocytes and lymphocytes, and furthermore that, in myeloid metaplasia, myelocytes and granulocytes may be derived directly from lymphocytes.

The second volume includes the sections on bone and cartilage, joints, muscle, the sense organs, and the nervous and reproductive systems. Here can be found one of the few satisfactory treatises in the current literature on bone and cartilage, written by P. G. Shipley. The major rôle of the osteoclast in bone destruction accompanying normal bone growth is questioned and subjugated to physio-chemical processes. Osseous changes resulting from different metabolic states are well outlined. T. Wingate Todd, describing the specialized systems of the heart, advances doubt as to the anatomical differentiation of the sino-auricular and sino-ventricular nodes from the remainder of the Purkinje tissue, and challenges the usual idea concerning the distribution of the latter in the heart. The ability of primitive Purkinje cells to develop into genuine myocardial cells is interesting, if true. A. T. Rasmussen advises more conservatism in assigning definite function to the interstitial cells of the testes from the present data, which he deems definitely insufficient for any sweeping generalizations.

In the opinion of the reviewer, the two volumes, consisting collectively of thirty-seven sections, comprise an admirable accomplishment, valuable to the advanced medical student or investigator, either in the general field of biology or medicine, both as a review, and as a reflection, to a variable extent, of the individual views and experiences of each contributor. Frequently, the direction which further experimentation and study may profitably take is indicated. Detail necessarily varies with the subject and the author, but the different divisions are in most cases adequately reviewed. But, as stated by the editor in his preface, the nature and scope of the publication necessitate certain limitations, such as the consideration of historical development. Nevertheless, a satisfactory bibliography appended to each section serves this purpose to anyone interested. The illustrations are for the most part photomicrographs and are highly satisfactory. A convenient index completes each volume.

W. L. W.

BOOKS RECEIVED

The Foundation of Health. A Manual of Personal Hygiene for Students. By WILLIAM BARNARD SHARP, S.M., M.D., Ph.D. Second edition, thoroughly revised. Illustrated. 1927. 8°. 288 pages. Lea & Febiger, Philadelphia. $2.50.

Pernicious Anemia. By BEAUMONT S. CORNELL, M.B. (Tor.), 1927. 8°. 311 pages. Duke University Press, Durham, North Carolina. $4.00.

Mental Health of the Child. By DOUGLAS ARMOUR THOM, M.D. Harvard Health Talks. 1928. 12°. 46 pages. Harvard University Press, Cambridge. $1.00.

Animal Biology. By J. B. S. HALDANE and JULIAN HUXLEY. 1927. 8°. 344 pages. At the Clarendon Press, Oxford. $2.50.

The Endocrines in General Medicine. By W. LANGDON BROWN, M.A., M.D., F.R.C.P. Modern Medical Monographs. Edited by Hugh Maclean, M.D., D.Sc., F.R.C.P. 1927. 8°. 144 pages. Paul B. Hoeber, New York. $3.00.